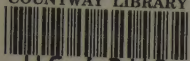


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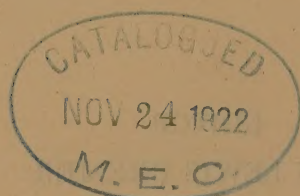
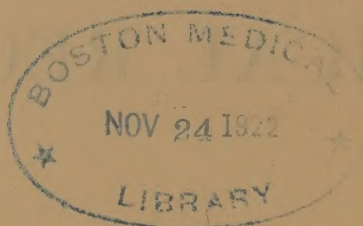
THE ANATOMICAL RECORD

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JOHN LEWIS BREMER
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Resumen por el autor, E. V. Cowdry.

Una comparación de las antiguas láminas anatómicas chinas con la "Fünfbilderserie" de Sudhoff.

Sudhoff y sus discípulos han descubierto una serie de cinco diagramas tradicionales de los sistemas muscular, esquelético, nervioso, venoso y arterial en las bibliotecas europeas y en manuscritos persas en las siguientes localidades; 1) Claustro de Prufening, Baviera, 1158 D. J.; 2) Claustro de Scheyern, Baviera, 1250 D. J.; 3) Biblioteca de la Universidad de Basilea, unos 1250 años D. J.; 4) Biblioteca Bodleiana, Oxford, 1292 D. J.; 5) Misma biblioteca, unos 1340 años D. J.; 6) Oficina de la India, Londres, antes de 1400 D. J.; 7) Raudnitz, Bohemia, siglo XIV. Aunque no idénticos, se corresponden en tal grado que Sudhoff está justificado al deducir que todos ellos provienen del mismo sitio, probablemente de Alejandria. Los esquemas chinos coleccionados por el emperador K'ang Hsi (1661-1722 D. J.) y atribuidos por él a la dinastía de Han (206 A. J. a 220 D. J.) se asemejan a las "Cinco series de figuras" de un modo general en lo referente a la forma y proporciones, y posición forzada del cuerpo. Son mas primitivas y antiguas. Si existe alguna relación entre ellas hay razones para suponer que las figuras chinas son el original, las cuales a causa de su antigüedad carecen de detalles.

Translation by José F. Nonidez
Cornell Medical College, New York

A COMPARISON OF ANCIENT CHINESE ANATOMICAL
CHARTS WITH THE 'FÜNFBILDERSERIE' OF
SUDHOFF

E. V. COWDRY

Anatomical Laboratory of the Peking Union Medical College

SIX PLATES (TWENTY-FOUR FIGURES)

After reading the MS. of Dr. Hsieh's "Review of Ancient Chinese Anatomy," Col. Fielding H. Garrison was kind enough to suggest to me that some of the diagrams illustrated may perhaps be related to the 'Five Picture Series' of Sudhoff. Generous help from Professor Hu Suh of the Government University from our instructor in Chinese, Mr. Ma Kiam, and from Dr. E. T. Hsieh, has made it possible for me to examine many Chinese drawings and to establish dates accurately.

I am also indebted to the librarian of the National Library (1) for the unusual privilege of being permitted to enter the library itself and to examine the originals personally. Through the kindness of Mr. R. F. Johnston, tutor to the Emperor (2), a card of introduction was obtained to Mr. Ch'i Ling (3), a minister of the household who with great courtesy introduced me to Mr. Fu Ch'i (4) who, in turn, allowed me to consult the private library of the imperial family in the Forbidden City. Mr. Ch'i also arranged visits to the office of the imperial physicians (5), which is maintained by grants from the emperor who receives yearly \$4,000,000 from the Republic as an honorarium in consideration of his partial abdication. Here 110 physicians are registered, none of whom have any foreign training. Two of them visit the court twice a week and make prescriptions when necessary. Their official text-book, the 'Golden Mirror' (6), is the most interesting book I have found. Attached to the office of the imperial physicians is a small temple where an image of Huangti, the father of Chinese medicine, is worshiped during the spring and autumn festivals.

Sudhoff's series of five traditional diagrams representing the muscular, skeletal, nervous, venous, and arterial systems, was discovered by him in different European libraries and in Persian MS. as follows:

The Cloister of Prüfening near Ratisbon, Bavaria, (Munich, 13002), 1158 A.D.

The Cloister of Scheyern, Bavaria (Munich, 17403), 1250 A.D.

The Basel University Library (Provençal MS., D, II, ii), about 1250 A.D.

The Bodleian Library, Oxford (Ashmolean, 339), 1292 A.D.

The Bodleian Library, Oxford (Codex 19) about 1340 A.D.

India Office, London (Persian MS. 2296), before 1400 A.D.

The library of Prince von Lobkowitz, Raudnitz, Bohemia, (VI, Fc, 29) 14th century.

Specimens of the Persian and Provençal series are reproduced in plate 1.

Although the drawings in the different series are not identical, they correspond so closely that Mortimer Frank is justified in saying (p. 53) that "It may be asserted with almost historic perspicuity, that these figures with their text must have been based upon a short illustrated Alexandrian text-book of anatomy which was written in Greek and provided with schematic drawings done probably after representations then extant." Sudhoff feels that they were originally drawn in Alexandria.¹ Whatever their source, they influenced the teaching of anatomy in Europe until human dissection came to the rescue, as it is now doing in China.

In more recent European drawings the squatting posture, characteristic of these diagrams, is lost, they have become, as Mortimer Frank remarks (p. 58), free of the "constrained posture of centuries and present an easy pose." The same author inserts the following footnote:

"Dr. Berthold Laufer, of the Field Museum at Chicago, discovered during his travels four Tibetan anatomic plates, which

¹ Through the courtesy of Colonel Garrison, I was at first able to examine Sudhoff's original papers in the Surgeon-General's Library, but since my return to China I have had to rely entirely upon Mortimer Frank's review of Sudhoff's work.

evidently belong together and are still in use by students in Tibet today. They recall, in their squatting posture, so many of the details of the Prüfening and Persian figures that one is forced to believe that the well-known post-Alexandrian anatomic figures were brought to India also and that the Indian and Chinese doctrines went through a peculiar process of amalgamation whose later results still confront us in the Tibetan plates."

Unfortunately, I have not had an opportunity to examine these plates, but Surgeon-General Chuan, who has spent two years in Lassa, tells me that he has seen a large number of Tibetan anatomical plates and believes them to have been introduced from China. Moreover, Heinrich Laufer (p. 11) calls attention to the fact that Tibetan historical classics, written in 630 A.D., record the derivation of Tibetan medicine from China.

The Chinese diagrams which I have to report bear a certain resemblance to the 'Five Picture Series' of Sudhoff, as may be seen by reference to plate 2 in which figures 5 and 6 from the Raudnitz series are compared with figures 7 and 8 from the 'Golden Mirror' which is a compilation by the Emperor K'ang Hsi (1661-1722 A.D.) of earlier works on anatomy attributed by him to the Han Dynasty (206 B.C. to 220 A.D.). The two diagrams illustrated are exact copies of drawings which I found in a MS. entitled 'Leiching' (7) written by Chang Chieh Pin (8) about 1500 A.D. in the library of the imperial family.

1. In both European and Chinese series the entire body is taken as the unit and is represented by a simple outline drawing with very little attempt at perspective.

2. The peculiar squatting posture is evident in both, though it is perhaps more marked in the European figures.

3. With characteristic Chinese sense of propriety, the external genitalia are omitted in both series.

4. In the Raudnitz drawings the feet are abducted (turned laterally) almost to the point of dislocation, as in Chinese classical drawings. This abduction is illustrated to a less extent in K'ang Hsi's figures.

5. The general proportions of the body are alike. The trunk is longer than the legs, as in infants. Relatively short legs

(femora), though of course not exaggerated to this extent, are a feature of some Orientals, particularly of the Japanese. As far as I can ascertain, with our limited library facilities, ancient Egyptian illustrations depict figures which, far from being stubby in appearance, have relatively long legs.

From a large number of other drawings I have selected figures 9 and 10 for further comparison with the Raudnitz series. They are taken from a book on acupuncture and moxa (9) by Yang Chi Chow (10) which was written between 1573 and 1627 A.D. and consists of a collection and critical digest of older works. It antedates the 'Golden Mirror,' but has not received the same official recognition. It will be noted that the feet are completely abducted and thus correspond more closely with those in the Raudnitz series. The arrangement of the traveling vessels varies, as compared with the 'Golden Mirror' figures, but the general proportions of the body are maintained with typical conservatism. Compare also figures 11 and 12 from a book by Hsia Ting (11) on 'Yin Ke Tih Ching' (12) written during the reign of the Emperor Tao Kuang (1821-1850 A. D.), and figures 13 to 16 from the 'Hsi Yuan Lu Pien Ching.'

Even if we suppose a genetic connection between certain Chinese anatomical diagrams and those of Sudhoff, it would perhaps be too much to expect a very close resemblance, owing to the great difference in time and space. Nevertheless, the correspondence between K'ang Hsi's illustrations (figs. 7 and 8) and the Raudnitz series (figs. 5 and 6) is almost as close as that between the Persian drawings (figs. 1 and 2) and the Provençal series (figs. 3 and 4). It is only when we come to compare the details of the arrangement of the vessels and nerves that the similarity breaks down. Sudhoff is able to support his contention that his different series spring from a common source by the discovery that a similar Latin text often accompanies them, but the idea expressed in the Chinese diagrams seems to be radically different from the Western conception.

Each of Sudhoff's series consists of at least five drawings representing the muscular, skeletal, nervous, venous, and arterial systems, for which few counterparts can be found in Chinese

literature. Chinese anatomists did not distinguish between arteries and veins, and very seldom specifically referred to nerves. The skeletal system is occasionally illustrated, as in figures 13 and 14, which are reproduced from a book by Chu Chang Yung called 'Hsi Yuan Lu Pien Ching' (13) said to be an exact copy of a Sung Dynasty (960-1127 A.D.) original. In figure 14, showing the skeleton from behind, the author, with the usual disregard of detail, is content to represent the areas in a schematic way, not troubling himself to illustrate the individual bones. This is particularly noticeable in the hands. I have been unable to find any diagrams of the muscular system. Perhaps this is explained by the circumstance that the Chinese as a race see nothing beautiful in the play of muscles in the body in motion. Their artists, from very early times, have devoted all their attention to color values and to the representation of draped figures. How different from Greece where, as Garrison and Streeter remark (p. 375), nudity was the 'festal costume'! The Greeks acquired their knowledge of musculature "not from dissection, but from empirical observation of athletes in action during games and military exercises." European artists took a very active interest in anatomy, as may be seen from the following statement of Vesalius, which I also quote from Garrison and Streeter (p. 394):

"As for those painters and sculptors who flocked around me at my dissections, I never allowed myself to get worked up about them to the point of feeling that I was less favored than these men, for all their superior airs."

What the Chinese lose in the variety of the systems illustrated they make up for by the extent to which they are still saturated with the doctrine of circulating 'humors.' In this respect they resemble other primitive peoples. Typical diagrams of this kind illustrating the course of the male and female principles have already been mentioned (figs. 7, 8, 9, and 10).

With regard to the squatting posture, one of my students, Mr. Li Ke Ming, has made the interesting suggestion that the use of the diagrams for so many hundreds of years as acupuncture charts may be the determining factor, since the needles are

usually inserted while the individuals are sitting down. It will be noted that the position of the patient awaiting treatment in figure 17 is almost identical with the position of the body in the Persian diagrams (figs. 1 and 2). The stretching out of the arm in figure 18 and the frog-like position of the legs in figure 19 also reveal important acupuncture spots and call to mind figures 3 and 4 from the Provençal series. On the other hand, the body is often shown in other positions in order to illustrate conveniently other acupuncture spots, so that this similarity in position may after all be a mere coincidence. It should also be remembered that Chinese artists are accustomed, in their classical portraits, to represent the knees widely spread apart and the toes turned outward.

Other less plausible explanations suggest themselves. Perhaps the diagrams were used long ago as charms to discourage innumerable evil spirits. A drawing reproduced from de Pouvoirville by de Zwaan is interesting. It represents a skeleton in the same squatting attitude which gives it a ferocious and terrifying appearance (fig. 20). The posture is more constrained than that of Persian drawings. It is Japanese (Stratz, p. 126) and relatively modern.

It is difficult to determine the actual age of the Chinese diagrams. They are usually attributed to the mythical Yellow Emperor, Huangti, who, according to tradition, ruled in China about 2696 B.C. Neuburger, however, in common with modern Chinese historians, is sceptical regarding this assumption. Unfortunately, we know very little of Huangti. E. T. Williams tells us that, according to de Lacouperie, he was the leader of a band of immigrants entering China from the west, and remarks that "while several traditions associate the name of Huangti with the Province of Kansu, he was probably born beyond the western boundary of China." According to Li Ung Bing (22), 'real authentic history' begins with the Chou Dynasty (1122-255 B.C.). Writing seems to have been invented considerably earlier, though Zelia Nuttall and Elliot Smith (p. 17) question whether the art of writing was known in China before the sixth century B.C. The Chinese classics tell us that writing became

so complicated that an attempt at standardization was made in B.C. 868 when 3300 characters were officially recognized (Ross, pp. 115, 117). They remain to us as 'seal characters' on bronzes attributed to the Chou Dynasty. In the opinion of sinologues, exact correspondence in dates extends back to August 29, 776 B.C., when the same eclipse was observed and recorded both in Europe and China (Hirth, p. 174). Since earlier dates may easily be determined by computation, it is fair to assume, with Hirth, that "we should date the commencement of the historic period, as far as the main facts are concerned, many generations before Yu-wang (781-771 B.C.), while making allowance for doubts in the chronology owing to the two-fold tradition."

I have been unable to obtain clear-cut evidence of the existence of any anatomical diagrams before the Han Dynasty, 206 B.C. to 220 A.D. The diagrams which I have illustrated are for the most part fairly recent prints of older originals. They can be traced back as follows:

Figures 7 and 8, Han Dynasty, 206 B.C.-220 A.D.

Figures 9 and 10, 1573-1627 A.D.

Figures 11 and 12, 1821-1850 A.D.

Figures 13 to 16, Sung Dynasty, 960-1127 A.D.

Figures 17 to 19, Sung Dynasty, 960-1127 A.D.

Figures 21 to 24, Sui Dynasty, 581-618 A.D.

It is not improbable, however, that they existed still earlier in the form of traditional sketches, emanating perhaps from central Asia, where Pumpelly has brought to light interesting remains, dating back, according to Professor Ulrich Duerst, of Berne, to about 8000 years B.C. A Western origin of the Chinese people is claimed by Père Richard, de Lacouperie, and others, and vehemently denied by Giles, Hirth, and Ross.

Since, however, Mortimer Frank has suggested that Tibetan anatomical plates, which closely resemble these Chinese diagrams, are derived from Sudhoff's hypothetical Alexandrian originals, we have to consider carefully the possibility of Egyptian derivation.

According to Elliot Smith, there is "sufficient information to justify the conclusion that many of the fundamental conceptions of Indian, Chinese, and Japanese civilization were planted

in their respective countries by the great cultural wave which set out from the African coast not long before the sixth century B.C."

In this connection it cannot be denied that the anatomical diagrams reproduced in plate 6 exhibit a distinctively Egyptian appearance. The profile view with the prominent nose is remarkable since it is so different from what we ordinarily meet with among Chinese. The nose in figure 21 seems to be almost semitic. Figures 22 and 23 illustrate perhaps the Lange 'law of frontality,' referred to by Garrison and Streeter (p. 372), for they gaze 'directly and rigidly forward,' as in Egyptian statuary. They are also disposed as in a bas-relief. Mr. Ma Kiam and Mr. Ch'i Ling inform me that the style of dress in figure 22 is certainly not Chinese. The skirt of figure 23 is suggestive of a sketch on one of the pillars of the Hypogeum of Seti I, as reproduced by Maspero (p. 148). The coiffure is apparently of about the Han Dynasty (Ma Kiam).

These four drawings are from a book by Hsi Fang Tzu (14) called 'Ming Tang Chih Ching' (15). This is a print of an original published in the Sung Dynasty (960-1127 A.D.) which I have examined in the National Library and found to be similar, at least as far as the drawings are concerned. They are often mentioned in Chinese history. 'Ming Tang' is, according to Professor Hu Suh, a generic term, of unknown origin, applied to practically all anatomical diagrams. The policy of the Emperor T'ai Tsung of Tang (627-649 A.D.) is said to have been influenced by an examination of the 'Ming Tang' diagrams. They are also mentioned in the bibliographical sections of the Histories of Sin (16) and of Tang (17). He is of the opinion that they have been employed as anatomical diagrams, illustrating the points of application of moxa, from the beginning and that they are not simply old originals recently used for this special purpose. The notation is unusual, because the reference lines do not indicate areas limited by small circles as in the other drawings which I have reproduced.

They may have been introduced into China by sea in the manner suggested by Elliot Smith. They may also have come overland, for, as Fenollosa remarks, Chinese military domination was

felt as far west as the Persian Gulf during the Han Dynasty, and rock carvings in Shantung caves made at this time display in his opinion, primitive Egyptian designs. It is not difficult to find other suggestions of Egyptian influence (Barbezieux-De Zwaan, p. 137).

Giles refers to some old stone sculptures collected for a mausoleum by the Wu family in 147 A.D., which are still the subject of considerable controversy. Douglas feels that they show unmistakable Egyptian influence, which, however, Paleologue and Chavannes deny (Giles, p. 12). Chavannes invokes the theory of 'convergence' in the following words:

"En fait, on découvra des rapports entre les premiers essais artistiques de tous les peuples parce que partout les mêmes causes produisent les mêmes effets; mais il faut se rappeler que, par un corollaire de ce même principe, ressemblance n'implique pas filiation."

Since the drawings are not, however, of the squatting type, they only influence our general problem by indicating the possibility of the Egyptian derivation of the squatting figures, for which I know of no other evidence.

It is possible that transference took place in the reverse direction, from China to Egypt, either overland or by sea. The Phenician mariners could hardly remain uninfluenced by their contact with the Orient. A few centuries later Arab traders (de Goeje) carried back, among other things, Chinese bottles containing classical quotations which may be identified (S. W. Williams) and from which I select the following:²

Wang Wai, 702-745 A.D.

Wei Ying-wuh, 831-837 A.D.

Su Tung-po, 1068-1085 A.D.

These and other 'cultural waves' may have influenced life and thought along the Nile at about the time that Sudhoff considers the originals of his 'Five Picture Series' to have been drawn. It is even possible that rough diagrams representing the body in

² Obviously, they were brought to Egypt after the dates mentioned and probably before the end of the Sung Dynasty (1127 A.D.) when the Arab trade with Canton and Hangchow commenced to decline.

the squatting position were introduced from China about this time, or earlier, which perhaps served as a basis on which the Alexandrians grafted their more accurate knowledge. According to Mr. Ch'i Ling, there is a tradition, hazy it is true and incapable of verification, but none the less insistent, that in the old days Chinese anatomical diagrams were sent to the peoples of the West. Certainly, the Chinese drawings are older and more primitive than the European and Persian series thus far discovered. If any relation exists between them there is every reason to suppose that the Chinese are the originals which by reason of their age are wanting in detail.

- 1 京師圖書館
- 2 宣統
- 3 耆齡
- 4 福啟
- 5 太醫院
- 6 醫宗金鑑
- 7 類經
- 8 張介賓
- 9 鍼灸大成
- 10 楊繼洲
- 11 夏鼎
- 12 幼科鉄鏡
- 13 洗冤錄 辨正
- 14 西方子
- 15 西方子明堂灸經
- 16 隋
- 17 唐
- 18 瞿中溶
- 19 宋淳祐 珠
- 20 瘡瘍經驗
- 21 竇漢卿
- 22 李文彬

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PLATE 1

EXPLANATION OF FIGURES

Examples of the 'Five Picture Series' of Sudhoff

1 Arterial system, from Persian MS., no. 2296, in India Office, London. (From Frank's translation of Choulant's history and bibliography of anatomic illustration. Originally from Sudhoff's *Geschichte der Anatomie in Mittelalter*, Leipzig, 1909, plate V.)

2 Venous system, from Persian MS. no. 2296, in the India Office, London. (Reproduced from same source as fig. 1.)

3 Arterial system, from a late thirteenth century Provençal MS. (D II ii) in the Basel University Library. (Reproduced from same source as fig. 1.)

4 Male generative system from a Provençal MS. in the Basel University Library (D II ii). (Reproduced from same source as fig. 1.)



ARTERIAL SYSTEM, FROM PERSIAN MS. NO. 2306, IN THE
INDIA OFFICE, LONDON

(From the Medical Library, 1900, Plate XIV)

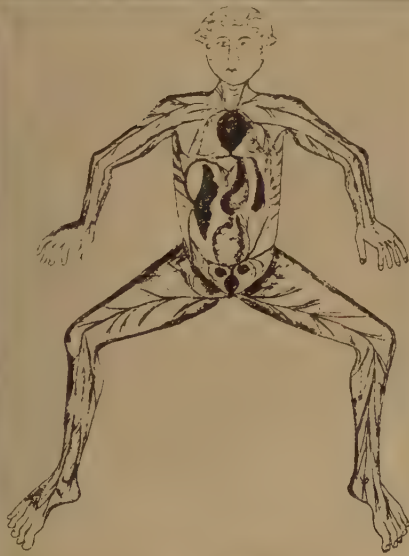
1



VENOUS SYSTEM, FROM PERSIAN MS. NO. 2306, IN THE
INDIA OFFICE, LONDON

(From the Medical Library, 1900, Plate XIII)

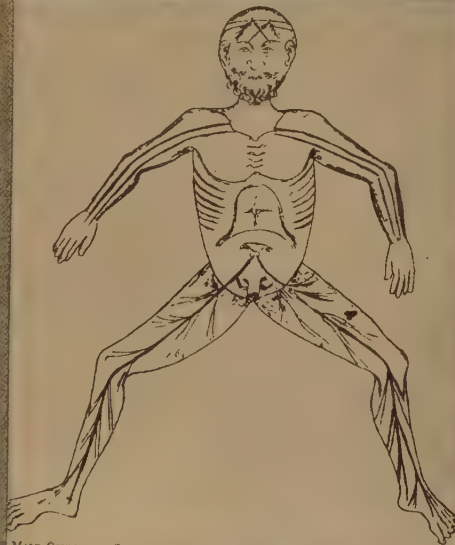
2



ARTERIAL SYSTEM, FROM A LATE THIRTEENTH-CENTURY PROVENÇAL MS (D. II. 41.)
IN THE BASEL UNIVERSITY LIBRARY

(From Sotthoff, Geschichte der Anatomie im Mittelalter, Leipzig, 1900, Plate V)

3



MALE GENITIVE SYSTEM, FROM A LATE THIRTEENTH-CENTURY PROVENÇAL MS (D. II. 41.)
IN THE BASEL UNIVERSITY LIBRARY

(From Sotthoff, Geschichte der Anatomie im Mittelalter, Leipzig, 1900, Plate IX)

4

PLATE 2

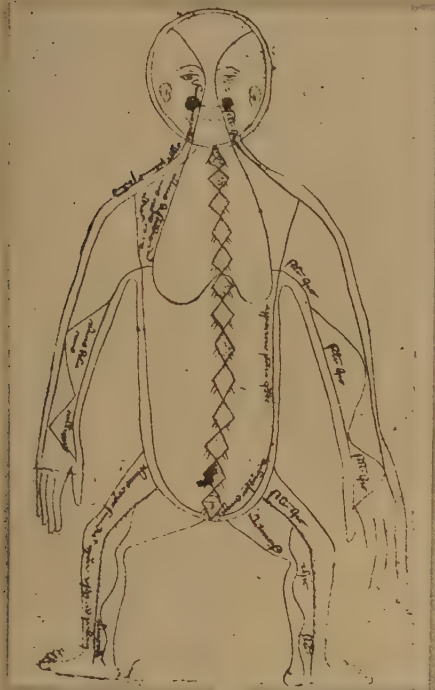
EXPLANATION OF FIGURES

Comparison of the 'Five Picture Series' (figs. 5 and 6) with drawings edited by the Emperor K'ang Hsi (figs. 7 and 8)

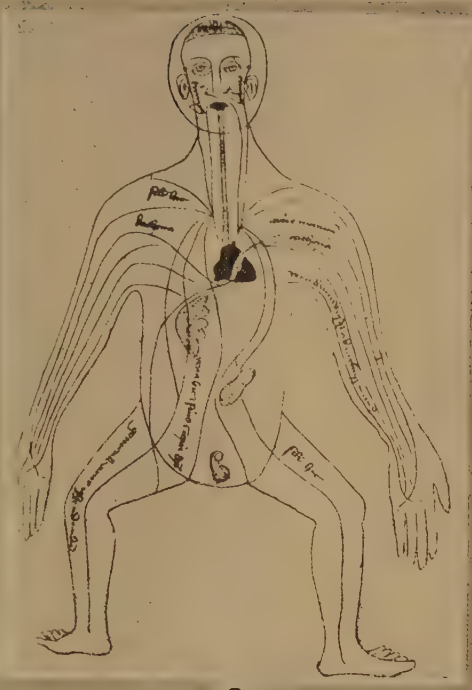
5 Nervous system, from a fourteenth-century MS., in the library of Prince von Lobkowitz (Raudnitz, Bohemia). Reproduced from Sudhoff: *Arch. f. Gesch. d. Med.*, Leipzig, 1909-10, Bd. 3, Pl. XI.

6 Arterial system, from a fourteenth-century MS. also in Prince von Lobkowitz's library, from Sudhoff: *Ibid.*, Pl. IX.

7 and 8 Diagrams of the course of the traveling vessels, after Hsieh. Compiled by the Emperor K'ang Hsi (6) (1661-1722 A.D.) from originals attributed by him to the Han Dynasty (206 B.C. to 220 A.D.).



5



6



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8

PLATE 3

EXPLANATION OF FIGURES

Examples of old Chinese diagrams

9 and 10 Diagrams illustrating the course of the traveling vessels from a book on acupuncture and moxa (9) by Yang Chi Chow (10) written between 1573 and 1627 A.D. The drawings are certainly copies of older originals.

11 and 12 Acupuncture charts from a book by Hsia Ting (11) entitled 'Yiu Ke Tih Ching' (12) written during the reign of the Emperor Tao Kuang (1821-1850 A.D.), probably derived from older works.



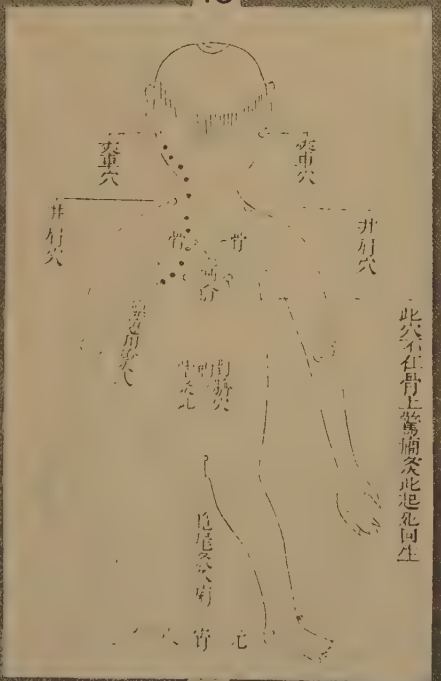
9



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PLATE 4

EXPLANATION OF FIGURES

Further examples of old Chinese diagrams

13 Skeletal system from a book by Chu Chung Yung (18) entitled 'Hsi Yuan Lu Pien Ching' on legal medicine (13), said to be a new and revised edition of a Yuen edition (19) of the 'Hsi Yuan Lu,' originally published in the Sung Dynasty (960-1127 A.D.).

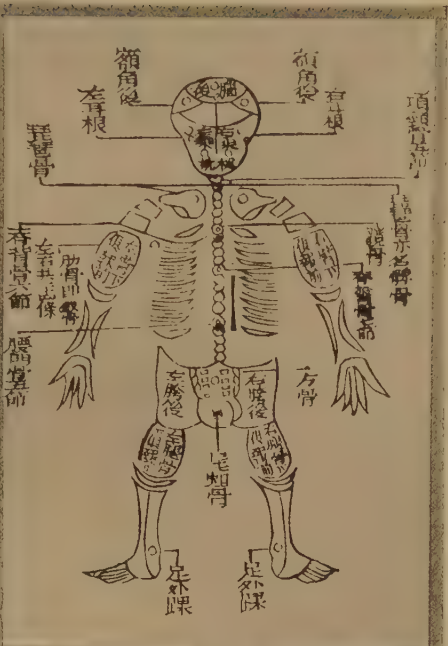
14 Skeletal system, same source. Having already illustrated the anterior view, the author does not feel it necessary to fill in the individual bones as seen from behind, so he merely represents the areas concerned in a schematic way.

15 Acupuncture spots, anterior view, same source.

16 Acupuncture spots, posterior views, same source. The black circles are shown in red in the original and are supposed to indicate places where mortal wounds may be inflicted.



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PLATE 5

EXPLANATION OF FIGURES

Drawings illustrating the possible origin of the squatting position

17 Patient awaiting treatment. From a book called 'Chuang Yang Ching Yen' (20), by Premier Do (21), of Sung, published during the reign of K'ang Hsi (1661-1722 A.D.), said to be a copy of a Ming edition of the Sung book. Note the similarity between this position and that shown in the Persian sketches (figs. 1 and 2).

18 Acupuncture diagram from a book by Chang Jea Ping (8), also a copy of a Sung original. Note how the position of the arm and hand corresponds with figure 6 of the Raudnitz series.

19 Acupuncture diagram from the same source in which the position of the legs is suggestive of the Provençal series (figs. 3 and 4).

20 Relatively modern drawing of a Japanese skeleton in extreme squatting attitude from de Zwaan.



17



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PLATE 6

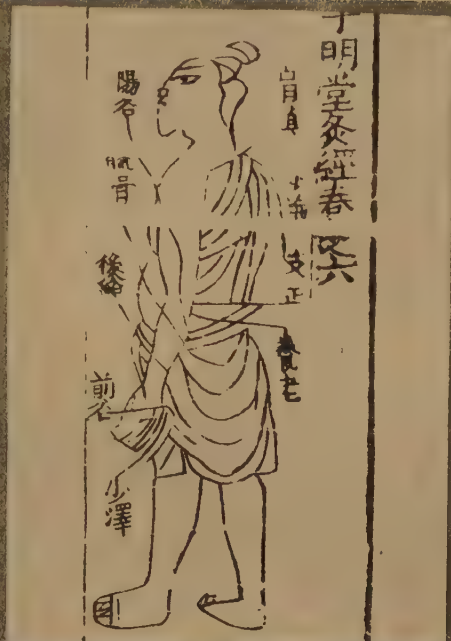
EXPLANATION OF FIGURES

Drawings suggestive of Egyptian derivation

21 to 24 Sketches from a book by Hsi Fang Tzu (14) called 'Ming Tang Chih Ching' (15), reprinted from an original published in the Sung Dynasty (960-1127 A.D.). They are frequently mentioned in Chinese history. 'Ming Tang' is, according to Professor Hu Suh, a generic term of unknown origin applied to anatomical diagrams in general. The policy of the Emperor T'ai Tsung (627-649 A.D.) is said to have been influenced by an examination of 'Ming Tang' diagrams. They are also mentioned in the bibliographical sections of the Histories of Sin (16) and of Tang (17) and probably date back to the Sui Dynasty, 581-618 A.D.



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Resumen por el autor, William Martin Smallwood.

Notas sobre una ternera con dos cabezas.

El presente trabajo es una descripción de las modificaciones estructurales a consecuencia de la duplicación de la cabeza. Una fotografía obtenida mediante los rayos X revela la notable semejanza de los dos cráneos, no solo en el carácter general de los huesos, sino en sus pequeños detalles estructurales. Los dos cerebros no son del mismo tamaño, difiriendo también en la distribución de las circumvoluciones y en la médula; están unidos entre sí en la región del puente y la médula oblonga. En las dos lenguas existe el mismo número de papilas circunvaladas en vez del número doble, como sería de esperar.

Translation by José F. Nonidez
Cornell Medical College, New York

NOTES ON A TWO-HEADED CALF¹

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TWO TEXT FIGURES

Wilder and Johnston have each, especially the former, described 'double monsters,' yet a brief note on another is desirable, as a more detailed record is possible in this case than in others of a similar kind.

On the 25th of December, 1920 a registered Holstein, Kate Koningen De Kol, gave birth to a two-headed heifer, one week short of term. The cow is owned by W. H. Kinney, Elbridge, New York.

The calf was large and well formed except for the double head. It lived seventeen hours and was able to move the jaws independently. When given milk through one mouth, the milk escaped from the other. This was due to the condition of the esophagus which was closed. It was impossible to force a small probe from the opening of the esophagus in the neck into the mouth, the closure being opposite the larynx. The mounted specimen is in the museum of the College of Agriculture, Syracuse University.

A dissection of the specimen revealed that the organs of the body were normal and that the blood vessels of the neck showed no marked variation until the head was reached.

Johnston ('01) concludes his study of thirteen two-headed snakes with the statement that the point of bifurcation of the vertebrae is more posterior than would be supposed from external examination. The skulls frequently appear united externally when in reality they are not. Reviewing the literature, Johnston also states that the evidence certainly shows that there was a doubling of internal organs caudad to the vertebral bifurcation.

¹ Contributions from the Zoological Department in the Liberal Arts College, Syracuse University, C. W. Hargitt, Director.

This is not the case in either the vertebrae or other organs in the specimen under discussion. There is a single larynx with two folds of the soft palate and a single epiglottis. There is a single hyoid chain of cartilages as in a normal animal. The cartilages of the large larynx are also normal and there is no indication of morphological variation in this organ. Although the bleating of the calf escaped from either mouth, the sound came from a single organ.

The four incisors on each of the four mandibles are present, well developed and symmetrically distributed. These simple temporary teeth have their shovel-shaped crown narrower than normal. This is more pronounced in the 'corner incisors,' each of which is nearly conical, suggesting their possible canine origin.

Four deciduous premolars are clearly defined and correspond in proportionate size to these same teeth in a normal calf. A section of a normal mandible permits one to note the nature of the roots and compare them with the x-ray photograph (fig. 1). The clearly defined mandibular canal is similar in each mandible, with the roots of the incisors and premolars showing the same orderly arrangement as in a normal jaw.

In the dicephalous lamb described by Miss Bishop the mandibles on the inner side of the two snouts are only half as long as the outer ones from symphysis menti to rami. Nor do these mandibles articulate with the temporal bone.

In this specimen there is very little difference in the length of the four mandibles. The two inner are more slender, but still articulate with the temporal bone, while the outer ones are distinctly curved and slightly longer.

Three deciduous premolars are present on each upper jaw and alike in minute details. The space where the first permanent molar will form shows as a light area immediately posterior to the third deciduous premolar. We were at first inclined to believe that there were two teeth lacking, but a study of several normal calf skulls shows a similar variation.

The two skulls are identical in general shape and size. They are attached by the fusion of the temporal bones, which are much thickened. In fact, the thickened roof of the skull was very apparent when removing the brain.

The x-ray picture reveals that the several sinuses are clearly defined and of equal extent, even to details. As nearly as I could judge, the two heads were joined to the vertebral column on the same axis. There is nothing to indicate from a study of the skulls that one of the heads is to be explained as parasitic on the other head.

The two tongues are united at their base 3 cm. in front of the epiglottis. Each tongue shows the characteristic dorsal eminence. The fungiform papillae are numerous and clearly defined. In a normal adult there are from twenty to thirty vallate papillae irregularly distributed over the posterior part of the dorsal eminence. On the right tongue, outer margin, there are nine vallate; in the inner margin seven; on the left tongue, outer margin, seven, inner margin four—a total of twenty-six in the two tongues. Unless there is a marked increase in the number of vallate papillae after birth, then these structures are not doubled, which constitutes an interesting exception and one difficult to explain.

The two brains are united in the region of the trapezoid and posterior medulla. When the occipital bones were removed, the dura mater was continuous over the cerebellar region and was very tough.

On one side there was a sac-like swelling, which suggested that one head only contained a brain, but this proved to be untrue as soon as it was cut open.

The falx cerebri was loosely attached in the longitudinal fissure of the right brain. In the left brain, it extended half-way to the corpus callosum.

The tentorium cerebelli of the two brains is connected by a strong sheet, which is much thickened about 25 mm. from the longitudinal fissure of the left brain. From this thickening to the same region in the right brain, the sheet becomes thinner. The left tentorium cerebelli is massive and solid, extending over the left surface of the cerebellum and crura as a greatly thickened tissue with massive fibers showing on the surface, which is in marked contrast to the delicacy of this same structure over the right brain, where no indications of thickenings are to be seen.

In removing the brains the dura mater over the right brain was found to be more delicate than over the left.

The more critically one studies these two brains, the more evident it becomes that the right one is not a complete duplicate of the left. In a general way it is smaller in the thickness of the cerebral hemispheres, in the conspicuousness of the temporal lobe, and in the detailed pattern of the gyri (fig. 2).

The median vertical surfaces of these brains bring out the difference more clearly. In the brain of the calf there is the gyrus cingulum just dorsal to the corpus callosum, a supracingular gyrus and a marginal gyrus. This marginal gyrus passes posteriorly into the occipital lobe; while the cingulum and supracingulum unite in the region of the genu of the corpus callosum to pass around to the hippocampal gyrus. The main variation in these gyri is found in the right half of the right brain where the supracingular gyrus projects 5 mm. into the median longitudinal fissure beyond the outer gyri. It is also much thicker where it usurps almost entirely the place of the cingulum, of which only a trace remains. These two gyri in the opposite half, and in both halves of the left brain, are similar in extent and contour.

The general pattern of the gyri and sulci of the cerebrum is quite variable in calves, at least in the ones used this year in class work in neurology. Without specifying the detailed variation of these structures, it can be said that there is a marked difference in this respect between the right and left brains. Each brain was compared with the brain from a normal calf, and it was easy to find one that exhibited a pattern of the gyri like the right brain and another like the left. The difference, then, is such as occurs in calves of separate parents.

There is also an interesting difference between the tracts of the olfactory and optic in the two brains. The piriform lobe is slightly irregular on the right half of the right brain. This irregularity consisting chiefly in the small size as compared with the other three olfactory lobes. In both brains, the two olfactory tracts on the surface (medius and intermedius) are prominent and of equal size. The intermedial tract just as it enters the piriform sends a small tract to the tuberculum olfactorium. Here all of the parts appear alike.

There is no apparent difference in the four eyes. Each is normal in size and external appearance. A well-formed optic nerve passed to the brain to cross in a definite chiasma. The median section of the brain revealed that the area of the section of the two chiasmata were very unequal, that of the left brain nearly twice as great. When the optic tracts are followed to their destination, those of the left brain continue as a broad, flat band to the superior colliculus, while the one on the right half of the right brain continues as a band for 15 mm. and then breaks up into two or three small bundles, the posterior one passes beneath some of the fibers of the cerebral peduncle, then coming to the surface and ending on the posterior margin on the superior colliculus, one strand of which enters the inferior colliculus. The optic tract of the opposite hemisphere is more like those in the left brain. Although each eye is normal, the optic nerve of one seems to have been disturbed during its growth in aberrant distribution of one of the optic tracts.

The four oculo-motor nerves are present, but slightly asymmetrical. A median section passing through the third ventricle leaves both nerves attached to the median half of each hemisphere. Each of the trochlear nerves was located in its normal relations, but the abducens could not be found.

As the two brains are joined from the region of the trapezoid to the posterior margin of the medulla, there is a great deal of confusion in these structures. In this region some injury was done to the right brain in dissection. The spinal cord was unmodified and more definitely united with the left brain. The fourth ventricle spreads out under both cerebella as a common cavity to the brachium pontis. The right wall of the medulla is much enlarged, having more than twice the thickness of the left, and from this region an irregular mass extends posteriorly about 2 cm. It is perfectly obvious that the right brain is dependent on the left, rather than each sharing equally a connection with the spinal cord. It would seem as if the right brain had failed to receive its full quota of embryonic nerve tissue, which cannot be said of the skulls.

The optic tract in the right brain certainly departs from the normal pattern—a condition which Miss Bishop says does not exist in the two-headed pig embryo. Whether it is proper to conclude that the gyri, especially the cingulum, also depart from the normal pattern is open to discussion. They are certainly not identical in the two brains.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 X-ray photograph of skulls and mandibles of two-headed calf. Note the similarity, even to minute details of tooth structure, mandibular canal, and sinuses which appear as light areas. Photographed by Dr. C. F. Potter.

2 The two brains with the remains of the dura mater showing in the longitudinal fissure and united over the cerebella. Notice that the detailed pattern of the gyri of the two brains is not alike. There is a posteriorly projecting mass beneath the right cerebellum, which together with other structural modifications suggests that the right brain is parasitic on the left. Photographed by Dr. C. F. Potter.



Resumen por el autor, A. R. Barnes.

La fascia pélvica.

El no haber reconocido la existencia de elementos estructurales en la fascia pélvica, cada uno de los cuales presenta diferencias en su desarrollo ha resultado en dos errores de descripción; el uso de los términos fascia visceral y parietal, es confuso y debe suprimirse. La descripción de las reflexiones de la fascia pélvica, que se consideran como una capa continua extendida sobre las paredes pélvicas y vísceras debe suplantarse por afirmaciones que indiquen donde se unen las unidades separadas de la fascia. La coalescencia de ciertas capas peritoneales contiguas en los fondos de saco prevesical y rectovesical y su transformación en fascia constituye una unidad estructural que está representada por las fascias umbilico-vesical y rectovesical. Las arterias umbilicales del embrión en las fases tempranas del desarrollo están rodeadas por una masa de tejido mesodérmico. Este tejido persiste en una forma condensada alrededor de estas estructuras, denominándose la vaina umbilico-vesical en el adulto. Del mismo modo, el tejido mesodérmico que envuelve a los vasos y nervios pélvicos, al recto y a la próstata persiste como en forma de vainas vasculares no organizadas que rodean a dichas estructuras. Otro grupo comprende las fascias relacionadas con los músculos, cuya extensión está determinada por la de los músculos con quienes están relacionadas. Con la excepción de la fascia de la superficie superior del músculo elevador del ano, estas fascias consisten simplemente del delgado perimysio que se forma sobre los músculos en las demás regiones del cuerpo. El diafragma urogenital está formado por los ligamentos arcuado y transversal pélvico y por un tabique fibromuscular bastante grueso. La superficie inferior del diafragma está envuelta por una fascia muy delgada.

Translation by José F. Nonidez
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THE PELVIC FASCIA

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EIGHT FIGURES (TWO PLATES)

INTRODUCTION

Much careful work has been done on the pelvic fascia. Almost every conceivable view of fascial arrangement has been advanced.

The following description is based on the development of the pelvic fascia, and has been found capable of demonstration in the class-room. The agreement between this description and the dissection has enabled the student to gain a clearer conception of pelvic fascia than has been possible from the accepted text-book description.

One of the greatest sources of confusion in the description of the pelvic fascia has been the attempt to show that it could be traced as a continuous layer over the entire pelvis and by some authors even over the perineum.

At the outset it is important to recognize that there are certain structural units of the pelvic fascia—units that can be accounted for by differences in development. The coalescence of certain contiguous layers of peritoneum and their subsequent replacement by connective-tissue lamina represents one type of fascia. The umbilicovesical and rectovesical fasciae belong to this first class. Secondly, we have the fasciae related to muscles; for example, the fascia covering the obturator internus, that covering the levator ani, and that of the perineal muscles. Lastly, we have that very considerable and much misunderstood mass of visceral mesodermal tissue surrounding the branches of the hypogastric arteries on their way to the viscera of the pelvis. If we keep these three structural units in mind, having as our object

the study of their arrangement, the question of continuity will become less important and will take care of itself.

This study has been confined to the male pelvis and perineum. Besides directing the dissection of some fifty-five pelvis in the laboratory, I have made twelve special dissections of cadavers, some injected with formalin, others in the usual way (equal parts of alcohol, glycerin, and phenol). Sagittal, coronal, and transverse sections of frozen cadavers have been freely used. Serial sections of the pelvis of human embryos of three, four, and five months were also studied. These sections were cut 20μ in thickness. Half the sections were stained in haematoxylin and eosin and the alternate sections in haematoxylin and picro-fuchsin.

THE UMBILICOVESICAL FASCIA

The extra peritoneal fatty tissue is commonly regarded as the only structure separating the fascia transversalis from the peritoneum. Over a triangular area, extending superiorly to the umbilicus and laterally to the obliterated umbilical arteries, two other structures intervene between the fascia transversalis and the peritoneum. These structures receive the name umbilicovesical fascia and umbilicovesical sheath in the adult.

Cuneo and Veau ('99) have shown the part played by the peritoneum in the origin of the umbilicovesical fasciae. A short account of their work will serve to make clear its formation and arrangement. They have shown in their study of this region that the middle part of the embryonic bladder, together with the umbilical arteries, is at first placed in contact with, or partially imbedded in, the anterior abdominal wall. The bladder and arteries soon disengage themselves from this wall and invade the coelomic cavity. A section through a human embryo 45 mm. in length shows this latter condition (fig. 4). The bladder and umbilical arteries are seen surrounded by a mass of mesodermal tissue, called the allantoic sheath. They are covered by peritoneum except anteriorly where there is a connective-tissue stalk, the mesocyst, joining their mesodermal sheath to the anterior abdominal wall. This leaves a pair of culdesacs (fig. 4) anterior to the allantoic sheath separated by the mesocyst. This meso-

cyst is represented at an earlier stage by a mere strand of tissue. Then the culdesac on either side is gradually obliterated by the fusion of the peritoneal layer on the posterior surface of the anterior abdominal wall with that on the anterior surface of the allantoic sheath (fig. 5). The fused peritoneal layers persist as a thin but fairly strong fascial stratum called, in extra-uterine life, the umbilicovesical fascia. A study of my series of 45- and 80-mm. embryos confirms this view, and figures 4 and 5 illustrate the process described above.

The general contour of the umbilicovesical fascia is triangular (fig. 6). Accordingly, it has two surfaces—a posterior and an anterior—an apex, a base, and two borders.

The apex of the umbilicovesical fascia corresponds to the umbilicus. Leaving the umbilicus, the borders gradually diverge from the median plane. Each lateral border of the fascia is attached to the anterior surface of the peritoneum along a line lateral to the obliterated umbilical arteries (fig. 6), an attachment not hard to understand in the light of the process illustrated in figures 4 and 5. Delbet ('01) states that the fascia follows the umbilical arteries in their oblique course laterally and downward, and that it is attached to the entire anterior border of the great sciatic notch. This seems to me somewhat theoretical. I have found the fascia thinning out rapidly on its inferolateral border and blending with the mesodermal tissue about the vessels of the bladder. Inferiorly and in the medial plane, we find the umbilicovesical fascia firmly adherent to the anterior surface of the bladder. It is possible in favorable cadavers to trace this fascia downward anterior to the bladder and demonstrate its attachments to the true ligaments of the bladder, the latter actually constituting the inferior attachments of the fascia in its medial portion (fig. 6). It is important to recognize that the base of the fascia is concave postero-inferiorly and that its concavity embraces the neck of the bladder.

UMBILICOVESICAL SHEATH

"The allantois arises as a tubular diverticulum of the posterior part of the yolk sac" (Lewis, '18), but subsequently is connected with the hind gut. McMurrich ('14) states that it

"pushes its way into the solid mass of mesoderm which forms the bellystalk." In an 80-mm. embryo we find the bladder and umbilical arteries imbedded in an extensive mass of mesodermal connective tissue (fig. 5), to which Cuneo and Veau ('99) have given the name allantoic sheath and for which a better name would be umbilicovesical sheath, inasmuch as the allantois is in the bellystalk and has no part in the formation of the bladder (Lewis, '18). Delbet ('01) states in this connection that the allantovesical sheath, preferably called umbilicovesical sheath, atrophies, becoming converted into a membrane stretching from the obliterated umbilical arteries laterally to the urachus superomedially, and the bladder inferomedially. This umbilicovesical sheath occupies a space bounded in front by the umbilicovesical fascia; posteriorly by the peritoneum and the rectovesical fascia (fig. 6).

Theoretically, the apex of the umbilicovesical sheath should reach the umbilicus in the adult. In practice one will hardly be able to trace it so high. It widens out as it descends, following for its outer borders the course of the obliterated umbilical arteries. Reaching the anterior angle of the flaccid bladder in the median plane, this membrane is continued over all the surfaces of the bladder forming its sheath. This covering has been erroneously described as coming from the fascia covering the upper surface of the levator ani. The lateral portion of the umbilicovesical sheath is continued downward past the lateral border of the bladder and blends immediately with the mesodermal tissue surrounding the vessels to and from the bladder.

It should be pointed out that the prostate is similarly developed in a mass of mesodermal tissue. This mesodermal tissue is later condensed about the prostate to form the prostatic sheath. This sheath joins the bladder sheath superiorly and blends with the superior surface of the urogenital diaphragm inferiorly. A small portion of the fascia on the superomedial surface of the levator ani is attached to the prostatic sheath laterally. Anteriorly, the sheath blends with the connective tissue of the preprostatic venous plexus, while posteriorly the sheath is joined by the rectovesical fascia.

THE RECTOVESICAL FASCIA

It has been pointed out how two layers of peritoneum in the prevesical culdesacs fused and were replaced by a fascia. A similar culdesac of peritoneum exists in the foetus between the bladder and seminal vesicles in front and the rectum behind. Usually a like culdesac exists between the bladder in front and the seminal vesicles posteriorly. Here, again, Cuneo and Veau ('99) have shown that these culdesacs are gradually obliterated by the fusion of the opposing peritoneal surfaces. The fused peritoneal layers persist as connective-tissue lamellae. The latter constitute the rectovesical fascia, one lamella separating the bladder and seminal vesicles, the other lamella separating the seminal vesicles from the rectum (fig. 7).

The rectovesical fascia has its superior attachment to the peritoneum as the latter leaves the rectum to pass on to the bladder (figs. 6 and 7). Extending downward between the bladder and rectum, the fascia has an inferior attachment to the sheath of the prostate gland immediately below the seminal vesicles. If this fascia is followed laterally, it will be found to blend very quickly with the mesodermal connective tissue about the vessels to and from the bladder. In figures 6 and 7 a fascia passing down in the interval between the bladder and seminal vesicle to attach to the prostatic sheath is clearly shown. I have not found this last lamina invariably present.

The rectovesical fascia is firmly attached superiorly to the peritoneum, so that an attempt to separate the two results in a tearing of the peritoneum. The peritoneum is usually slightly furrowed, due to this attachment.

 SOME ANOMALIES IN THE DEVELOPMENT OF THE UMBILICO-
VESICAL AND RECTOVESICAL FASCIAE

The peritoneal culdesacs, seen in the 45-mm. embryo, sometimes persist in the adult. In one dissection I found these peritoneal sacs persisting. They were located, as they are in the embryo, anterior to the umbilicovesical sheath, and each extended almost to the median line.

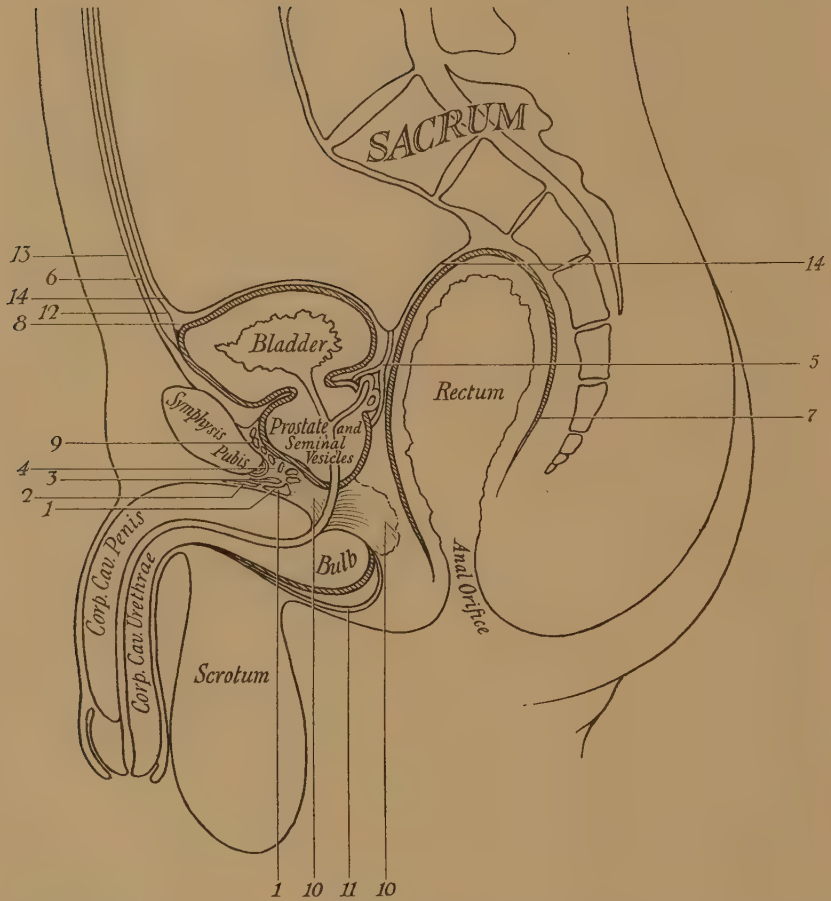


Fig. 1 Sagittal section of human pelvis through symphysis pubis (modified after Gray). Diagrammatic. 1, Ligamentum transversum pelvis and 2, its anterior lamina; 3, dorsal vein of the penis; 4, ligamentum arcuatum pubis; 5, rectovesical fascia; 6, umbilicovesical fascia; 7, condensed mesodermal tissue clothing rectum; 8, condensed mesodermal tissue of umbilicovesical (allantoic) sheath clothing the bladder; 9, puboprostatic ligament and venous plexus; 10, fibrous septa forming superior layer of urogenital diaphragm; 11, fascia covering bulbocavernosus muscle; 12, the umbilicovesical (allantoic) sheath; 13, fascia transversalis; 14, peritoneum.

In another dissection, the opposing peritoneal layers of the culdesacs had fused laterally in the neighborhood of the umbilical arteries. Failing to fuse medially, a pair of closed peritoneal pockets remained to remind one of the embryonic condition.

Similarly, the two peritoneal layers which fuse and later are replaced by the rectovesical fascia sometimes fail to unite. In one dissection I found such a condition in which the peritoneal pocket extended downward to the prostate, its lowest part separating the seminal vesicles and rectum.

Such anomalies as these substantiate the interpretation advanced above as to the origin of the umbilicovesical and rectovesical fascia, for the variations are exactly what one might predict, having the embryological condition in mind.

THE LEVATOR ANI AND ITS FASCIA

In a comparative study, Peter Thompson ('01) has found the iliococcygeus muscle arising from the iliopectineal line. The same muscle in man arises at various levels on the inner surface of the pelvic wall. Derry ('07) has found fibers of this in man reaching up into the neighborhood of the iliopectineal line—a condition the writer has encountered in two human pelvis. Derry ('07) believes, therefore, that when the muscle falls short of this line, it is replaced by aponeurotic remains, an inference that my dissections repeatedly confirm. This aponeurosis is independent of the fascia covering the inner surface of the obturator internus muscle (fig. 2).

If the levator ani be dissected from below, it will be seen to arise from the inferolateral surface of this aponeurosis. Accordingly, as Derry ('07) has suggested, above the level of origin of the iliococcygeus muscle from its aponeurosis is an area of the pelvic wall clothed with two fascial elements, viz.: *a*) the aponeurosis of the iliococcygeus and, *b*) the thin fascia covering the obturator internus. This area is limited posteriorly by the anterior border of the greater sciatic foramen.

The upper surface of the levator ani is clothed by a distinct fascia usually well described. Delbet ('01) gives perhaps the

most exhaustive and precise account of its extent. The inferior surface of the muscle is clothed by a very delicate sheath, which, if called fascia, ought to be recognized as a thin perimysium. Superiorly, the portion of this perimysium related to the pubococcygeus muscle joins the aponeurosis of that muscle, while anteriorly the part of the perimysium related to the pubococcygeus muscle joins the pubic bone and its ascending ramus. Inferiorly the perimysium joins the anal fascia (fig. 3).

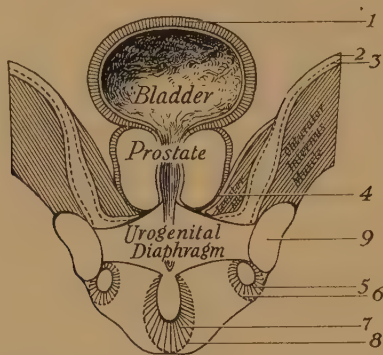


Fig. 2 Schematic drawing of coronal section of pelvis through prostate (modified after Cunningham). 1, bladder sheath; 2, aponeurosis of levatorani muscle; 3, perimysium covering inner surface of obturator internus muscle; 4, blending of fascia on upper surface of levator ani with sheath of prostate; 5, ischio-cavernosus muscle and, 6, its perimysium; 7, bulbocavernosus muscle and, 8, its perimysium; 9, descending ramus of pubis.

Two lines are frequently encountered on the upper surface of the levator ani. The more constant of these is the arcus tendineus fasciae pelvis. It stretches from the postero-inferior border of the pubic bone and symphysis to the ischial spine. The writer regards this as a thickening of the fascia which covers the upper surface of the levator ani muscle. It serves to fix the bladder and prostate to the wall and floor of the pelvis. Derry ('07) states here: "In this way the pubo-prostatic and lateral true ligaments of the bladder are formed." I believe his interpretation entirely correct. This line serves to join intimately the fascia of the superior surface of the levator ani with the bladder sheath and has been the basis for the erroneous

conception that the bladder is covered by a fascia formed by a reflection of the levator ani fascia.

Another line less frequently present is the arcus tendineus levatoris ani. It stretches across to the ischial spine from a point on the descending ramus of the pubis anterosuperior to the obturator canal. Text-books give this line as the origin of the iliococcygeal portion of the levator ani—a statement that



Fig. 3 Schematic drawing of coronal section of pelvis through acetabulum. (modified after Cunningham). 1, mesodermal tissue clothing colon; 2, aponeurosis of the levator ani muscle; 3, perimysium or fascia of obturator internus muscle; 4, lamina of rectovesical fascia splitting to enclose seminal vesicles and, 5, split portions enclosing seminal vesicles; 6, fascia on upper surface of levator ani; 7, perimysium, or fascia, on lower surface of levator ani; 8, supratsegmental space; 9, lamina terminalis fossa ischiorectalis; 10, fascia lunata (here called Alcock's canal); 11, ischiorectal fossa; 12, obturator internus muscle; 13, levator ani muscle.

my dissections only partially bear out. In 40 per cent of cases examined with reference to this particular point, the levator ani had this origin. In 60 per cent of cases the arcus tendineus levatoris ani was absent and the levator ani took origin from the arcus tendineus fasciae pelvis.

The anterior fibers of the levator ani run above the urogenital diaphragm to obtain insertion into the perineal body. The fascia covering these fibers inferiorly is in contact with the superior surface of the urogenital diaphragm and is firmly attached to it.

The obturator fascia and the aponeurosis of the iliococcygeus join the periosteum just below the iliopectineal line. The iliac fascia also attaches to the periosteum at or just above the iliopectineal line. If we speak of the iliac fascia passing over the brim of the pelvis minor to become continuous with the obturator fascia and the aponeurosis of the iliococcygeus, we must recognize that the continuity is effected through their mutual attachment to the periosteum along the iliopectineal line.

The extent of the obturator fascia is determined by the extent of the obturator internus muscle, inasmuch as the fascia clothes its inner surface and has attachments at or a little beyond the attachments of the muscle. Wherever the obturator muscle is in contact with the urogenital diaphragm, its fascial covering is attached to the superior surface of the diaphragm.

It must be mentioned that the obturator fascia is ordinarily a very delicate membrane. It is, in the opinion of the writer, an ordinary perimysium for the obturator internus.

THE PERINEAL FASCIA

The first layer of fascia encountered in the ordinary dissection of the perineum is the superficial perineal fascia. It consists of two parts. The first part, the superficial portion, contains fat and is continuous with the superficial fascia over the thigh and gluteal region. The second layer, the deep portion of the superficial perineal fascia is commonly referred to as Colles' fascia, the extent of which is usually accurately described. The connective-tissue sheaths of the bulbocavernosus, superficial transverse and ischiocavernosus muscles blend with Colles' fascia on its deep surface. These muscle sheaths in turn blend with the deeper-lying inferior fascia of the urogenital diaphragm. It is through these muscle sheaths, then, that Colles' fascia becomes indirectly connected with the inferior fascia of the urogenital diaphragm—a point not made clear by text-books.

The urogenital diaphragm is ordinarily considered to consist of superior and inferior fascial layers, enclosing the deep transverse perineal muscle, the sphincter of the membranous urethra, together with branches of the pudendal artery and nerve. The

urogenital diaphragm will be better understood if dissected in accordance with the following description:

The urogenital diaphragm may be considered as composed of several units. Anteriorly, the first of these is the arcuate ligament—a structure well described in the best text-books. Next in order, posteriorly, comes the transverse ligament of the pelvis. It is separated from the arcuate ligament by the dorsal vein of the penis and, in turn serves to separate that vein from the dorsum of penis. The deep or posterior part of the transverse ligament blends with the fibrous tissues surrounding the preprostatic venous plexus. Still farther posteriorly is the third and most extensive element, a strong fibromuscular septum. Its thickness, vertically, varies from 4 to 8 mm. The urethra pierces this fibrous septum and branches of the pudendal vessels and nerves enter its posterior border and pass through it anteriorly. Posteriorly, it is attached to the perineal body; laterally, the attachment is to the inner surfaces of the descending rami, and superiorly it blends with the sheath of the prostate (figs. 1, 2, and 7).

Superficially, these three units are covered by the inferior fascial layer of the urogenital diaphragm which occupies the interval between the posterior border of the transverse ligament of the pelvis and the posterior border of the superficial transverse perineal muscle. The bulb of the penis rests upon the inferior surface of this fascia, covering the greater part of its central portion. The perimysium surrounding the bulbocavernosus muscle joins the inferior fascia, the latter gaining attachment laterally to the medial edges of the ischiopubic rami. Delbet ('01) has emphasized this attachment and remarks: "This layer (the inferior fascia of the urogenital diaphragm) is to my mind less an aponeurosis than a ligament of the bulb which it fixes so solidly." I do not share this belief. The inferior fascia is so thin that veins can be seen through it, and were it not for its intimate connection with firm deeper-lying structures its support of the bulb would be far from 'solid.' Deep to the inferior fascia are the vessels and nerves to the bulb, together with the deep transverse muscle and the sphincter of the membranous urethra (figs. 1, 2, and 7).

THE MESODERMAL SHEATHS OF THE PELVIC VESSELS, PELVIC COLON, AND RECTUM

The student is usually much confused when trying to dissect the tissue about the pelvic vessels in such way as to conform to accepted descriptions of the pelvic fascia. The same difficulty arises in the dissection of the tissues about the colon. In this connection, Delbet ('01) says: "Ombredanne ('00) has clearly shown that some analogous sheaths (analogous to the vesical sheath) exist about the principal viscera; they support the *vessels* which go to the organs, whence the name vascular lamina, which he has given them."

Sections of my embryos show that not only the pelvic vessels, but likewise the pelvic viscera to which they are distributed are developed in the midst of loose mesodermal tissue. In adult life we find these mesodermal sheaths condensed about these structures. Therefore, the connective-tissue coverings about the vessels and viscera which they supply are continuous with each other. The sheath covering the pelvic colon and rectum is very vascular and is so closely adherent to the gut wall that it cannot be dissected away as a continuous layer. Because of its vascularity and looseness, it does not resemble what we ordinarily call fascia. But we must recognize that such a sheath is present and must understand its continuity with the vascular sheaths. We can then avoid the false conception that the fascia related to the muscles of the pelvic floor is reflected to cover the rectum and lower portion of the pelvic colon.

The sheaths about the vessels and viscera are not condensed to compactness. But this will be recognized as the most practical arrangement, when it is recalled that the distention of the bladder and colon must be provided for. Fascia, inelastic as it is ordinarily considered to be, could hardly serve as a sheath for organs varying so much in size.

THE FASCIA LUNATA

The fascia lunata, a term introduced by Derry, is a sheath for the pudendal vessels and nerves. It can be demonstrated about these structures as they leave the pelvis through the greater sciatic foramen and may be traced to the urogenital diaphragm.

The fascia lunata has a slight attachment to the sacrospinous ligament, but it is very firmly attached to the inferomedial border of the anterior portion of the sacrotuberous ligament.

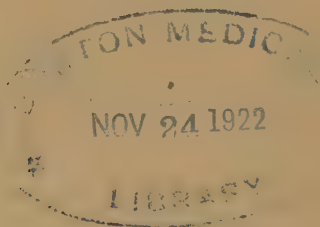
The fascia then comes into relation with the inferomedial surface of the obturator internus muscle. In this connection Smith ('07) says: "It (the fascia lunata) consists of an investment of fibrous tissue which has nothing to do with the sheath of the obturator internus muscle and is attached to the bony and ligamentous structures quite independently of it. It often happens (in the case of the human pelvis) that the fascia lunata becomes attached to the surface of the sheath of the obturator internus but this does not always happen."

The fact that we find quite as distinct a fascia lunata anteriorly and posteriorly where it has no relation to the obturator sheath supports Smith's ('07) view that it is not derived from the obturator sheath. However, all my dissections have shown the thin obturator sheath joining the fascia lunata (fig. 8), the latter gaining attachment to the sacrotuberous ligament, to the ischial tuberosity and to the ischiopubic ramus at a point about 1 cm. anterior to the fusion of the ischial with the pubic ramus.

The fascia lunata is commonly supposed to stop where the internal pudendal vessels and nerve enter the deep perineal pouch. It is no more deficient here than we saw it to be posteriorly as it emerged from between the greater and lesser sciatic ligaments. Instead of stopping, the sheath is broken up to be prolonged about the branches of the vessels and nerve in their passage through the perineum, thus constituting a considerable contribution to the urogenital diaphragm. Smith ('07) states that the fascia in the form of these sheaths actually becomes stronger because it affords an attachment to many fibers of the triangular ligament.

The portion of the fascia lunata which is related to the obturator internus muscle is commonly known as Alcock's canal. Smith ('07) has called attention to a septum passing from this portion of the fascia lunata to the perimysium on the lateral wall of the anal muscles. He calls this septum the lamina terminalis fossae ischiorectalis (fig. 8). I have been able to confirm the presence of this septum. It is important to note that this forms the roof of the ischiorectal fossa. This revises our view of the fossa which we have been accustomed to consider as extending upward to the junction of the fascia on the lateral surface of the levator ani muscle with the obturator fascia. The ischiorectal fossa is also limited anteriorly. It ends in several blind pockets filled with fat (fig. 8). These pockets may be regarded as being formed by a dipping downward of the lamina terminalis ischiorectalis. Smith ('07) points out that this arrangement keeps the ischiorectal fossa from extending forward above the urogenital diaphragm.

I am indebted to Dr. B. D. Myers for many helpful suggestions and for placing at my disposal abundant material for this investigation.



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PLATE 1

EXPLANATION OF FIGURES

4 Transverse section of 45-mm. embryo just above the symphysis pubis. Microphotograph. x10. 1, embryonic bladder and mesodermal connective tissue; 2, umbilical artery surrounded by mesodermal connective tissue, 3; peritoneal culdesacs separating the bladder from the rectus abdominis muscle; 4, the mesocyst; 5, rectus abdominis muscle; 6, peritoneum.

5 Transverse section through 80-mm. embryo just above the symphysis pubis. Microphotograph. X 10. 1, umbilical artery surrounded by much mesodermal connective tissue; 2, embryonic bladder surrounded by mesodermal connective tissue; 3, line along which the opposing peritoneal surfaces fuse, obliterating the peritoneal culdesacs of figure 4; 4, rectus abdominis muscle; 5, peritoneum.

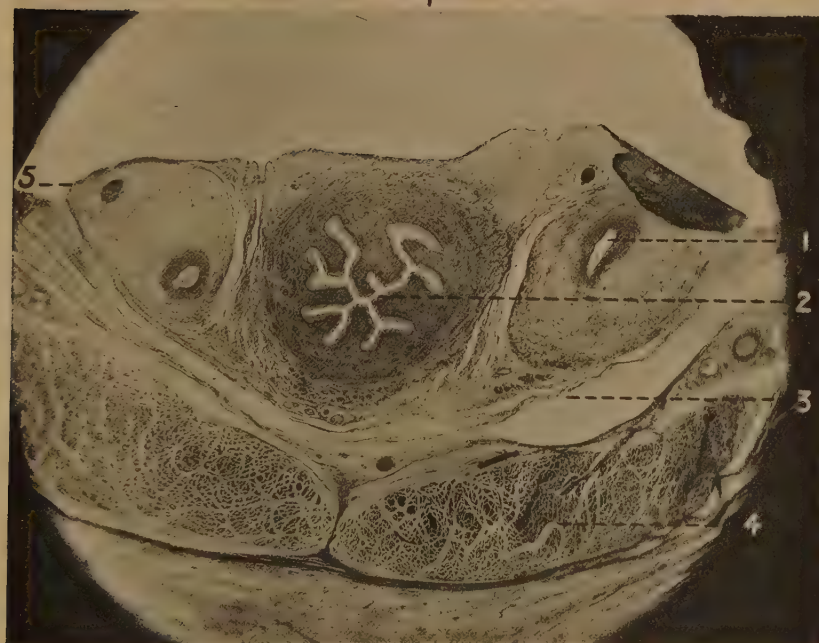
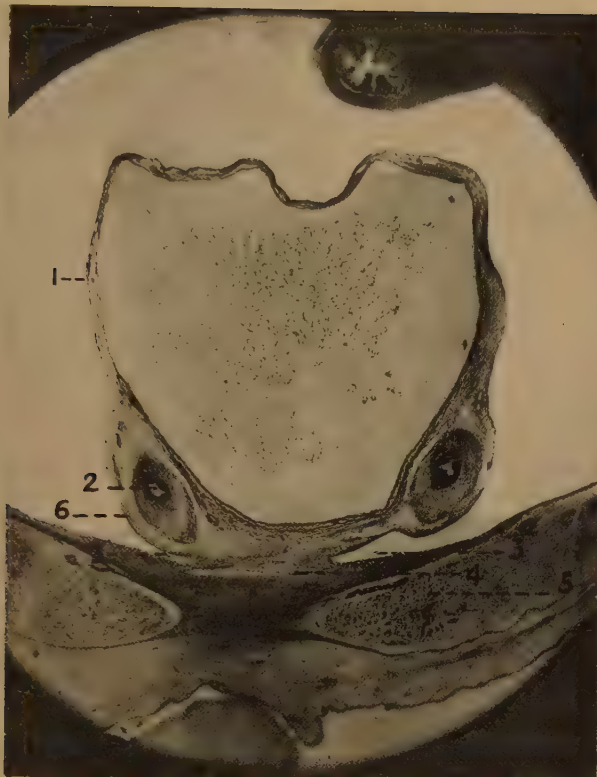


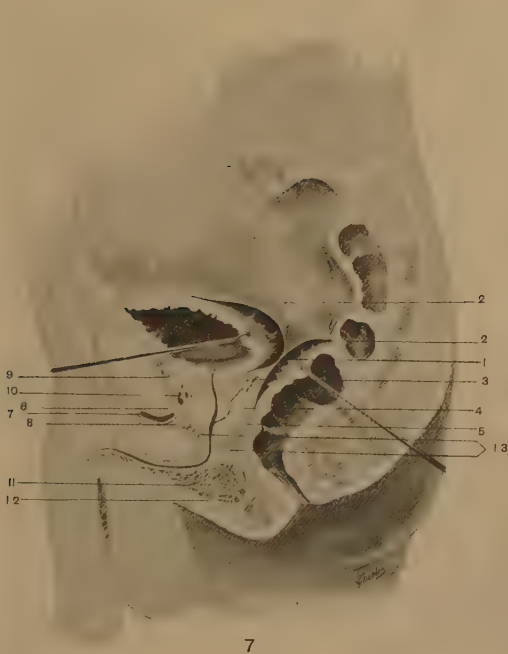
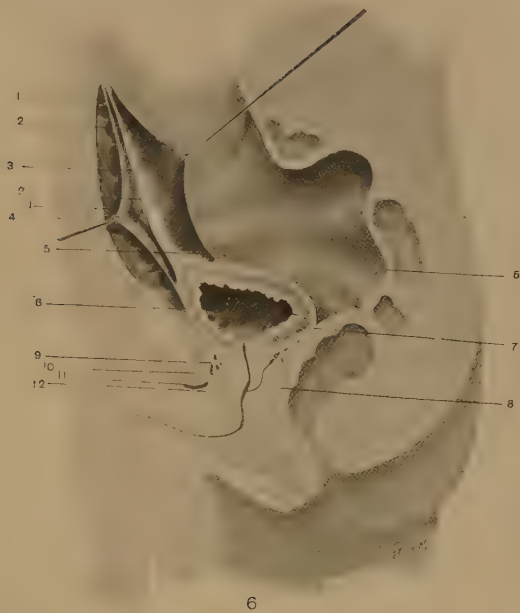
PLATE 2

EXPLANATION OF FIGURES

6 Sagittal section through symphysis pubis. 1, umbilico vesical fascia; 2, umbilicovesical (allantoic) sheath; 3, obliterated umbilical artery; 4, fusion of umbilicovesical fascia with anterior surface of peritoneum; 5, peritoneum; 6, attachment of umbilicovesical fascia to anterior true ligament of the bladder; 7, rectovesical fascia; 8, attachment of rectovesical fascia to capsule of prostate; 9, preprostatic venous plexus; 10, ligamentum arcuatum; 11, dorsal vein of penis; 12, ligamentum transversum pelvis.

7 Sagittal section through symphysis pubis. 1, rectovesical fascia; 2, peritoneum; 3, seminal vesicles; 4, prostate glands; 5, capsule of prostate; 6, ligamentum arcuatum; 7, dorsal vein of penis; 8, ligamentum transversum pelvis and anterior lamina; 9, retropubic fat; 10, preprostatic venous plexus; 11, capsule of bulb of penis; 12, perimysium of bulbocavernosus muscle; 13, fibrous septum making up a portion of superior layer of urogenital diaphragm.

8 Coronal section through anterior portion of ischio-rectal fossa. 1, aponeurosis of levator ani muscle; 2, levator ani muscle; 3, fascia lunata; 4, lamina terminalis fossae ischio-rectalis; 5, supratagmental space; 6, blind pockets in anterior portion of ischio-rectal fossa (fat removed).



Resumen por G. Carl Huber, por el autor, Wilder G. Penfield.

El aparato de Golgi y su relación con el trofospongio de Holmgren en las células nerviosas. Comparación durante retispersión.

El autor revisa brevemente las investigaciones sobre el aparato de Golgi y el sistema de canales del trofospongio de Holmgren, prestando particular consideración a la idea, mantenida por ciertos autores, de que el aparato de Golgi y el trofospongio de Holmgren son la misma estructura en las células nerviosas, que se manifiestan de modo distinto según los métodos de fijación y teñido, que dan imágenes positivas y negativas de una misma estructura. Usando la misma célula y mediante métodos sucesivos de teñido, el autor ha observado que no se encuentran canales que correspondan con el aparato de Golgi demostrado previamente, y que durante la retispersión, el retículo de Golgi se desplaza hacia la periferia celular, mientras que la posición intracelular de los canales de Holmgren no se altera. Ambas estructuras pueden demostrarse sucesiva o simultáneamente en el citoplasma de una misma neurona.

Translation by José F. Nonidez
Cornell Medical College, New York

THE GOLGI APPARATUS AND ITS RELATIONSHIP TO HOLMGREN'S TROPHOSPONGIUM IN NERVE CELLS. COMPARISON DURING RETISPERSION¹

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SEVEN FIGURES

INTRODUCTION

The intracellular relationship of the Golgi apparatus and Holmgren's trophospongium has been a subject of considerable dispute. Observations made by the writer while studying nerve cells in conditions of varying functional activity may throw some light on this problem. A preliminary brief review of the literature has been added, inasmuch as the work done on the comparison of the two structures has not been summarized in English and interesting contributions have been made since the excellent general reviews in German and Spanish by Duesberg and Cajal.

A reticular apparatus was first described by Camillo Golgi in 1898, in the cytoplasm of neurones in the central nervous system (21). In the same year Verati (44) described a similar structure in sympathetic ganglia. Outside of the nervous system, Pensa (40) was the first, demonstrating in the following year an internal reticulum in the cell cytoplasm of the suprarenals. Negri (37), also a pupil of Golgi, noted it in other ductless glands. After these pioneer observations, a large number of cytologists have observed the structure in cells of many types.

There has been considerable confusion as to the nomenclature of the structure in question. It has been variously called the internal reticular apparatus of Golgi (21), the endocellular reticular apparatus, the Binnennetz of Kopsch (33), the reticular

¹ From the Laboratory of Physiology, Oxford University. This communication formed part of a thesis accepted for the degree of B.Sc.

apparatus of Golgi-Holmgren (Cajal, 7), the canalicular apparatus (Bensley, 3), Golgi apparatus, etc.

Beginning in 1899, Holmgren (24) described an intracellular system of canals ramifying in the cytoplasm of neurones and certain other cells. He at once urged the identification of this canalicular structure, which he named 'trophospongium,' with the Golgi apparatus. Since this time the question of the identity of the two structures has been a subject of much discussion.

LITERATURE

Distribution of the Golgi apparatus in nature

A voluminous literature describing the apparatus in various types of cell has accumulated since Golgi made his original observations on spinal ganglion cells. The form of the apparatus varies somewhat from one type of cell to another, but common staining and developmental characteristics are exhibited throughout.

Cajal, in 1903, was the first² to demonstrate the Golgi apparatus in the nerve cells of invertebrates. His observations have been greatly extended for nervous and other tissue cells in all types of invertebrates by many workers, especially in the school of Nusbaum (38). Gatenby (19) states that "every sort of metazoan cell carefully examined, has been found to possess the typical apparatus" of Golgi.

Cajal (8) states that the reticular apparatus is well differentiated between the thirty-second and thirty-fourth hour of incubation in the germinal cells of the primitive streak (chick embryo). During ontological development, the reticulum and attraction sphere occupy the pole of the cell directed toward the external world, that is, the direction of cell migration or growth. According to Marcora (35), it is not until the tenth or twelfth day that the Golgi apparatus first appears in *adult* form *about* the cell nuclei of the more highly differentiated tissue. In cell division, Gatenby (20) has observed the Golgi apparatus (which is in the form of little rods lying on the archoplasm) to divide

² See Duesberg (16), p. 13.

itself equally between the two daughter cells. He concludes that "every mitosis or karyokinesis is also, as well, a dictyokinesis or nearly equal sorting out of Golgi rods between the daughter cells."

Bensley (3) described a typical intracellular reticulum in plant cells, staining with osmic acid, and watched it *intra vitam* break down from a living 'canalicular apparatus' to the 'multiply vacuolated' condition usually seen in the dead plant cell. This would seem to indicate that for plants, too, it may be possible, with improved technique, to demonstrate a reticular or canalicular cytoplasmic structure.

Thus the Golgi apparatus has been described as a cytoplasmic constituent of all types of cells in the animal kingdom, has been observed developing in the early stages of the embryo, and has been seen to grow and divide with cell multiplication.

Nature of the Golgi apparatus

The appearance of the structure varies somewhat from one type of cell to another, but in general it always occupies the cytoplasm of the cell and never encroaches upon the nucleus nor the cell periphery. In neurones it enters some distance into the dendrites. Its form is, in general, that of an irregular network.³ In nerve cells the meshwork is typically studded with lacunae, which sometimes appear full of impregnatable material and at other times empty, the boundaries *only* being outlined by the deposited silver or osmium.

With regard to the consistency of the apparatus, Cajal (8) has carried out some experiments which are of interest. In order to determine whether the 'materia argentofilia' was a liquid (and easily diffusible) substance or a semisolid substance not easily dislocated, he tore and crushed areas of spinal cord and brain. Upon subsequent microscopic examination, he found that at a tenth of a millimeter from the injured surface the Golgi apparatus

³ Workers in the Institute of Nusbaum have shown (38) that in the ganglion cells of cephalopods the Golgi apparatus takes on the form of threads which rarely join or cross. Likewise, in gastropods, Crustacea, and some insects the apparatus departs somewhat from its usual reticular arrangement.

within the cells was quite normal. On the very borders of the lesion or at a short distance from it were cells whose internal reticulum was broken, but replaced by tiny globules distributed throughout the cell, quite in accordance with the usual structure of the reticulum. There was no seepage nor fusion into large drops of the component material of the reticulum. His conclusion was that the material of the Golgi apparatus was viscous or semisolid and incapable of diffusing irregularly through the interstices of the neurofibrillar skeleton.

Concerning the structure of the apparatus, Cajal's theory, which resembles that of Holmgren in some respects, is briefly as follows: As seen in its most highly developed state in nerve cells, but also in other cells of the body, it is made up essentially of two different factors, a stable system of communicating spaces, and "a finely granular, vacuolated substance composed of complex material, endowed with great chemical and physiological activity—a mixture of lipoids (substances reduced by osmic acid) and of cytoproteids (with special affinity for silver)." The quantity and chemical composition of this mixture vary within wide limits according to the functional activity of the cell. This complex chemical is organized, during life, into granules or microscopic 'protomeras' endowed with the capacity to grow and, in multiplying, to bring about or assist at the chemical synthesis indispensable to cellular life.

This theory is broad enough to explain the varying morphology of the Golgi apparatus at least in normal conditions, as demonstrated by the variety of methods in use.

Functional activity of the Golgi apparatus

That the apparatus plays some essential rôle in cell life can hardly be questioned, inasmuch as it has been found generally in the cells of all types of living organisms. Moreover, there exists a proportional relationship between the normal cellular activity and the size and complexity of the Golgi apparatus. For example, the most complete and complicated reticulum is found in adult nerve and muscle cells. The function of these two classes of cells is, of course, associated with a great discharge of energy.

In 1909, Golgi (23) observed in the mucous glands of the stomach of a frog the displacement and change in form of the reticular apparatus corresponding to the varying functional activity proper to the cells. Kolster (32) similarly noted dislocation and metamorphosis in the goblet cells of the stomach accompanying the formation of secretion, and Tello (43), in studying sebaceous and mammary glands, described two phases through which the Golgi apparatus passed in active cells: hypertrophy and dispersion into fragments or droplets.

Cajal (8), in the goblet cells of the intestine, showed that in the resting stage the Golgi apparatus was small. With the beginning of the formation of secretory products, the apparatus becomes much hypertrophied. Before discharge of the cell contents, the apparatus itself becomes fragmented and partially passes out with the secretion. He showed that in odontoblasts, and osteoblasts as well, there is hypertrophy of the reticulum with the onset of cell activity.

Basile (2) has made interesting observations on the renal epithelium of the remaining kidney in animals after unilateral nephrectomy. The apparatus, which normally is found between the nucleus and lumen of the tubule, becomes hypertrophied, passes down about the nucleus, and at the end of the twelfth day is found at the base of the cell. Addison (1) noted hypertrophy of the reticulum of the basophilic cells of the anterior lobe of the pituitary after castration.

Weigl (45), after considering the changes occurring in the Golgi apparatus of gland cells, concludes that no light is thrown upon the actual function of the structure in those cells. The changes, he supposes, are due to mechanical displacement. But Duesberg (15) has reported changes in staining reaction of the Golgi apparatus during cell secretion and absorption which, in his opinion, indicated a qualitative as well as a quantitative change.

As to the actual function of the Golgi apparatus in the cell, Nusbaum (38), in reviewing the work of Weigl and the other investigators in his institute, concludes that the nature of the activity of the apparatus is nutritive (also Cajal, 8) and that it shows, typically, phases of increase and decrease associated with

this activity. Duesberg (16) and Sjövall (42) are unable to reach any conclusions as to function, and agree with Golgi (23) that "Relativement à la signification de l'organe reticulaire en question, . . . il s'agit d'un probleme dont la solution reste encore à trouver."

Functional changes in the Golgi apparatus of *nerve cells* were reviewed in a previous communication⁴ (39), and a specific reaction of the apparatus in these cells to axone section was described. This reaction consists in a dispersion of the intact reticulum to the periphery of the cell, *retispersion*, which may or may not be followed by *retisolution*. The question arises as to whether this added phenomenon throws any light on the function of the Golgi apparatus.

Retispersion after axone section is usually associated with chromatolysis. The nucleus may therefore be found at the cell periphery as well as the reticulum. If the conclusion of Cajal is correct, that the Golgi apparatus is of a semisolid consistency, then in retispersion associated with chromatolysis the two cell constituents which are displaced to the periphery are of more or less rigid form. This might argue that they were passively displaced from the center of the cell, *without* necessarily signifying any alteration in the function of either structure.

From the form of the apparatus in nerve cells (a system of lacunae joined by canaliculi), its variations (sometimes appearing hollow and at others full of impregnable material), its resistance to alteration in abnormal conditions (such as tetanus and pathological states generally, or separation from its connecting neurones by decerebration or cord section), it may well be an endocellular storehouse or circulatory structure. That is, a structure of more or less rigid form, the lipid and proteid content of which varies during normal cellular activity. Its function is yet obscure.

⁴Since this was written I have had the opportunity of seeing a very interesting demonstration of changes in the Golgi apparatus of nerve cells in the spinal cords of rats following exposure to cold. Demonstration—Congrès de Physiol., Paris, July 16-20, 1920—by C. Da Fano. It is learned from this investigator that a preliminary note on the subject will appear shortly.

Methods of demonstration of the Golgi apparatus

The methods by which the internal apparatus of Golgi can be demonstrated in nerve cells have become numerous. Briefly they are:

1. Modern method of Golgi (22). Fixation in formol, alcohol, and arsenious acid, followed by impregnation with 10 per cent silver nitrate. Reduction in any developer. The sections are toned with gold chloride.

2. The Cajal method (8, 10). Fixation in formol and uranium nitrate, impregnation with silver nitrate, followed by reduction in a solution of formaldehyde, hydroquinone, and sodium sulphite. Da Fano (13, 14) has modified this procedure by the substitution of cobalt nitrate for the uranium nitrate in the fixative with good results.

3. Kopsch method (33). Immersion of fresh tissue in 2 per cent osmic acid for eight days.

4. Besta (5) stained the apparatus with thionin after fixation in formol and acetic aldehyde, and followed by mordantage in ammonium molybdate.

5. Sjövall fixes tissue in formalin followed by immersion in osmic acid.

In addition to the above well-known methods, the apparatus is occasionally stained by many of the more general methods or may be seen unstained against a darker background (12). It may be stained *intra vitam* with neutral red or janus green (18). It has been demonstrated to me by J. B. Gatenby in fresh unstained cells of the ovotestis of *Helix aspersa* when they were teased out in the tissue juice.

A description of the methods employed in this study appears below.

Mitochondria (chondriosomes) and Golgi apparatus

Most recent writers are agreed that mitochondria and Golgi apparatus are separate structures, although possibly having some genetic relationship (Duesberg, 17, Cajal, 8; Cowdry, 12; Gatenby, 18; Nusbaum, 38). On the other hand, Monti (36) has recently

maintained that in adult nerve cells the mitochondria and Golgi apparatus are one and that the apparent difference is due to the different technic employed. It is true, that in procedures designed to demonstrate each structure, the other may occasionally appear as well, but this apparently is only an indication that the two structures have some common chemical constituents.

HOLMGREN'S TROPHOSPONGIUM

In 1899 Holmgren (24) drew attention to processes from outside entering the cytoplasm of the spinal ganglion cells of *Lophius piscatorius* and anastomosing there. In a postscript to this publication he cited Golgi's description of 'un fine apparato fibrillare' in the cytoplasm of the ganglion cells of mammals, and expressed the belief that the structure demonstrated by Golgi was only an intracellular part of the network of 'Saftkanälchen' which really communicated with the exterior.

In 1900 Holmgren (25) extended his observations to the spinal centers and ganglia of cats, rabbits, dogs, etc., using Carnoy's fluid principally for fixative, and iron hematoxylin or toluidin-erythrosin for stains. He showed that the 'Saftkanälchen,' or system of canaliculi, opened into the pericellular tissue spaces ('perizellulären Lymphspalten').

After these first descriptions he elaborated a somewhat complicated theory (26, 31) to explain variations in the appearance of the structure under consideration, which is briefly as follows: There is a network in the cytoplasm of certain cells to which he gave the name of 'trophospongium.' During cell activity, drops of liquid may be formed in the substance of the trophospongium thread. These drops coalesce, thus forming a canal within the thread, and this procedure may go far enough to dissolve the whole trophospongium, replacing it by trophospongium canals, the above-mentioned 'Saftkanälchen.'

In spinal ganglion cells he showed continuity between trophospongium and the enveloping subcapsular cells. In fact, according to Holmgren's view, the branching outgrowths of these enveloping cells or trophocytes, after entering the cytoplasm of the nerve cell, constitute the trophospongium. These ingrowths

possess the power of ameboid movements and their function is to take part in the nutrition of the neurone.

He proposed dividing all cells into two classes (26); *a*) a more highly differentiated class of cells all of which contain a trophospongium-canal system, and *b*) supporting cells, whose cytoplasm contains no trophospongium, but whose outgrowths, entering the cytoplasm of the cells of the first class, form a trophospongium there. Examples of this second class are subcapsular cells and neuroglia.

To sum up in Holmgren's own words (31, p. 135): "Summa summarum: ich glaube noch jetzt gültig Gründe zu haben . . . dass ein gewisser Inhalt des Netzes durch Umsetzungen in mehr oder weniger hohem Grade verflüssigt werden kann, wodurch Kanälchen entstehen, die durch azidophile Konturen, die den Resten der eigentlichen Fädchen entsprechen, abgegrenzt werden. An den Stellen des Netzes, wo die Dissolution zustande kommt, entstehen Kanälchen, die weiter sind als die übrigen Netzteile."

Numerous authors (Bethe, Cajal, Cowdry, et al.) have verified the existence of canals, *at least* within the cell.⁵

The question of the identity of the Golgi apparatus and Holmgren's trophospongium

In substantiation of his claim that trophospongium may be identified with the Golgi apparatus, at least in spinal ganglion cells, Holmgren presents in a recent article (31) excellent microphotographs of the Golgi apparatus blackened by the Kopsch method and, for comparison, iron-hematoxylin preparations in which the trophospongium canals stand out unstained in the ground cytoplasm. The general distribution of the two structures is the same and their form, although incompletely shown, is similar. Such appearances have led many authors to state that one structure is simply the negative picture of the other.

⁵ For an excellent summary of the work done on this point, see Duesberg (16), p. 47 et seq.

Such arguments are open to criticism, however. The two structures are being studied in cells of the same category, but *not in the same cell*. Also, even though they are frequently found occupying the same general location in the cell, their identity is not thereby established. Legendre (34) demonstrated similarity of general location for Golgi apparatus and Nissl granulations. But he did not thereby succeed in establishing that the two substances were identical.

Most workers have not been able to demonstrate extracellular connections for the trophospongium. In fact, Holmgren himself admits that all observers who have used only the osmic-acid or silver-impregnation methods, with the exception of Retzius and Smirnow, have concluded that the intracellular reticulum does not reach the surface of nerve cells. Sjövall (41), in ganglion cells, showed that it approached the subcapsular cells, but he could not establish true continuity.

In the opinion of Duesberg (16, 17), Nusbaum and his pupils, and also of Cajal (8), Holmgren, in maintaining the communication of the trophospongium with the exterior, has been confused by the appearance of exogenous processes which penetrate into (Duesberg, Nusbaum, Weigl), or make facets on (Cajal) the cell, without becoming continuous with the reticular apparatus.

Bensley holds that Holmgren's figures of intracellular nets in continuity with subcapsular cells stained by fuchsin do not prove continuity of structure, but more probably only a common affinity for the dye.

In a number of previous publications and in an admirable monograph ('15), summarizing the previous work, Cajal (8) has agreed that the trophospongium and Golgi apparatus in nerve cells were different representations of the same structure. The system of canals demonstrated by Holmgren, by Sjövall (41), and recently by Bensley (3), Cowdry (11, 12), et al., are produced, according to Cajal, not by a liquidation of the trophospongium, but by the fixatives used, partly or completely dissolving the reticulum. The occurrence of the canals would indicate failure of fixation of the reticulum or failure to stain it. The impregnation of the Golgi apparatus implies a fixation of the substance of

the reticulum and the deposition upon it of a metallic precipitate from the osmic acid or silver-nitrate solution.

Duesberg (16, p. 60), after a careful summary of the literature, concludes that the trophospongium, at least in nerve cells, cylindrical epithelium, and young oöcytes is identical with the Golgi apparatus in those cells. His conclusion was based on similarity of form and the changes undergone by the two structures at various ages of the animal. It should be noted here, however, that the development in the canalicular system has not been worked out as it has for the Golgi reticulum. Whether the structure appears as a set of canals or a compact network depends, in his opinion also, as in that of von Bergen, Bensley, Cowdry, et al., on the action of the reagents used.

The view taken by von Bergen (4) is somewhat different. He maintained that canals within the cytoplasm of ganglion cells are of two kinds. Type 1 resembles the Golgi apparatus in form and distribution and is really the negative picture of that structures. Canals of type 2 connect with the periphery of the cells, have no relation to the Golgi apparatus, frequently open into canals made by the microtome knife, and are in truth no more than artifacts. Canals of this type can never be stained, according to von Bergen. This theory has found few adherents. Holmgren's illustrations frequently show structures well stained which correspond to what von Bergen described as belonging to type 2.

Golgi and his school, on the other hand, refuse to consider the two structures as in any sense identical, although the former observed that some of the forms described by Holmgren are in some manner allied to the reticular apparatus. That the trophospongium and Golgi reticulum cannot be the same structure is, in the opinion of the Italian school, self-evident from the great difference in the form of the two structures. In agreement with the Spanish workers, they consider the reticular apparatus to be completely impregnated by the silver methods. The extra-cellular connections, therefore, as demonstrated by Holmgren, appear to the Italian school as additional evidence of the independence of the two structures.

Bethe (6) and Kopsch (33) likewise denied that the trophospongium is the same as the Golgi reticulum. The latter, using his entirely new but very simple osmic-acid technique, demonstrated an intracellular structure or 'Binnennetz' which corresponds very evidently with the Golgi reticulum and which, according to him, has no relation to trophospongium and does not show extracellular connections.

In considering the pros and cons of this much-debated question, it appears that the first group maintains that the Golgi apparatus and trophospongium-canal system are the same because of *similarity* of form and intracellular location. The larger part of this group deny the existence of extracellular prolongations of the canalicular structure (Cajal, Duesberg, Weigl, Bensley, Cowdry, et al.). The second group (Golgi and his pupils, Kopsch, et al.) deny that the two structures are the same because of *dissimilarity* of form. Much of the confusion arises from the variety of methods used, but also from the fact that Golgi apparatus and trophospongium have not been studied in the same cell carefully and that there has been no easily recognized alteration of one of these structures which could be evoked at will.

It is the object of this communication to draw the attention of cytologists to a method of successive as well as simultaneous demonstration of the Golgi apparatus and Holmgren trophospongium in the same cell, and to make a brief comparison of the two structures in neurone cytoplasm during retispersion subsequent to axone section.

METHOD

For this study the spinal cord and ganglia of cats were used, the Golgi apparatus being demonstrated by Cajal's formol-uranium-silver method, with certain minor modifications (39). After being stuck to the slide, the sections were counterstained by immersion in a dilute solution of Unna's polychrome-methylene-blue for from one to four hours. This was followed by passage through graded alcohols and differentiation in absolute alcohol. The sections were then mounted in Canada balsam,

and the result was a clear demonstration of Golgi apparatus and Nissl bodies, the latter appearing either green or blue, according to the intensity of silver impregnation in the cells. By this method the trophospongium canals may be demonstrated also. In some cells they will be found to stand out with great distinctness depending on the degree of differentiation. But I have found that these canals may be demonstrated more consistently and completely in the following way:

Drawings are made of the Golgi apparatus in certain selected cells (Cajal preparation). The cover-slip is then removed by immersion in xylol and the slide passed through downward graded alcohols to a 5 per cent solution of iron alum (Heidenhain), where it remains from twelve to twenty-four hours. This removes all silver from the cells as well as counterstain, and at the same time mordants the tissues for further staining. The sections are then treated as is usual in Heidenhain's iron-hematoxylin method. Great care is required to secure the proper amount of differentiation of the particular cells already drawn. At times it is necessary to mount the section in xylol and examine with high-power oil immersion, in order to be sure of the optimum differentiation of the Holmgren canals in the cell to be studied.

With the aid of the camera-lucida drawing previously made, it is easy to locate the same cell and to compare the two structures carefully. It is well to keep the nucleolus in focus during both examinations. Passing the sections up and down through the alcohol series must not be too abrupt, as otherwise a pericellular space may appear, especially in the spinal-cord sections. If a drawing was made of the canals demonstrated by the polychrome-methylene-blue in the original preparation, they will be found to coincide with those demonstrated now by iron hematoxylin in the same cell. Hematoxylin shows the canalicular structure usually in greater detail. It is evident that this staining is not influenced by the previous impregnation with silver, as there is no difference in the appearance of the peripheral as compared with the central cells. In the Cajal method the silver impregnation penetrates only a short distance below the surface of the block of tissue. No silver will be found in the central cells if the block is large.

If the section be left in the iron-alum solution for shorter periods of time, it is possible to mordant the tissue without removing the silver from the Golgi apparatus. The two structures then appear simultaneously in the same cell after staining with hematoxylin.

GOLGI APPARATUS AND HOLMGREN TROPHOSPONGIUM COMPARED DURING RETISPERSION

As mentioned above, after interruption of the axone there is a specific alteration in the distribution of the Golgi apparatus within the corresponding nerve cell, *retispersion* (Penfield, 39). As the name indicates, this alteration is a displacement of the net away from the center of the cell to the periphery. The axone base is also left clear of the apparatus (fig. 1). After this change there may or may not succeed a stage of dissolution of the Golgi apparatus, *retisolution* (fig. 2).

That the canals demonstrated by iron hematoxylin after removal of the silver as described above are really the same structure as that demonstrated by Holmgren, under the name of trophosphongium, is apparent after comparison of his figures⁶ with those presented here (e.g., 25, p. 86, pls. I and II; 27, p. 324, pls. XXV and XXVI; 29, p. 380, fig. 2).

We may now examine the Golgi apparatus and trophosphongium in the same cell under conditions which we know produce an alteration in the normal location of the former. The question arises whether or not in retispersion the Holmgren canals will likewise assume a peripheral distribution within the cell. If the two branching structures are identical, as certain authors maintain, one would expect to find both displaced to the cell periphery.

Such, however, is not the case. In figure 1 the Golgi apparatus (in black) was drawn with a camera lucida in a spinal ganglion cell impregnated with silver and without counterstain. Reti-

⁶ Whether or not these canals have any association with the neuroglial fibers which a number of authors have described penetrating the neurone body need not be discussed here further. It is also beyond the scope of this paper to discuss the possible relationship between the trophosphongium and the so-called trophocyte. The attention will be confined to the cytoplasm of the nerve cells.

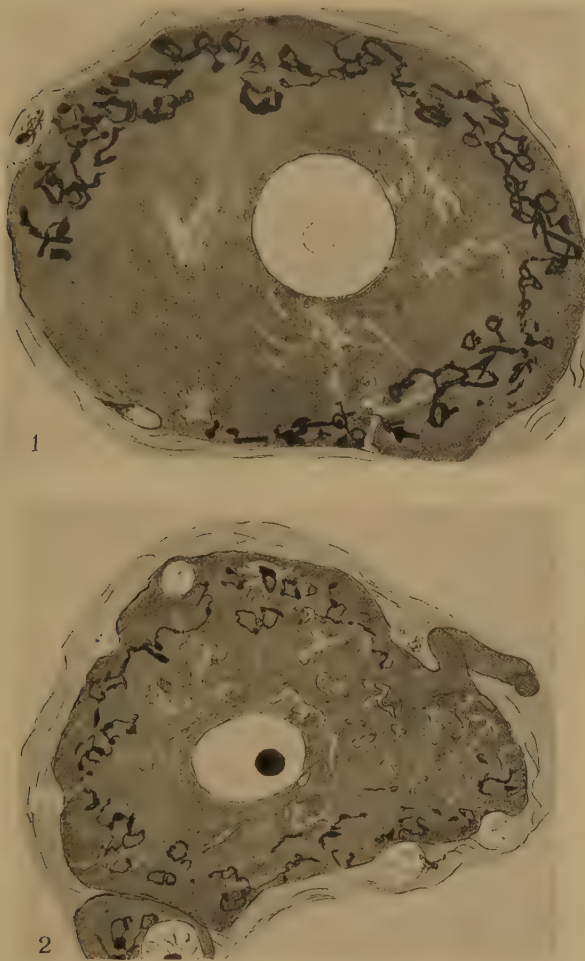


Fig. 1 Spinal ganglion cell of cat four days after section of the corresponding sciatic nerve. Retispersion evident. Successive staining method used (the Golgi apparatus and cell drawn from a Cajal silver preparation, the Holmgren canals are added after subsequent staining of the same cell with iron hematoxylin). Drawings made with Abbé camera lucida, Zeiss. Objective homog. immers. 1.5 mm., eye-piece no. 6 comp.

Fig. 2 Spinal ganglion cell of cat seven days after section of the corresponding sciatic nerve. Retispersion and retiresolution evident. Successive staining method as in figure 1.

spersion had begun throughout the ganglion, the sciatic nerve of that side having been cut four days previously. After removal of the silver, followed by staining with iron hematoxylin, the image of the same cell was superimposed upon the previous drawing by means of the camera lucida and the Holmgren canals drawn. It is apparent that the canals have not been forced to the cell periphery, as was the Golgi apparatus, under the influence of axone section, but have maintained the position occupied by them in normal cells (see also fig. 2).

If the canal system were the negative image of the Golgi apparatus, one would expect that drawings of a cell undergoing retispersion, treated in turn by the two methods described above, would show the same network, black in one drawing and unstained in the other, provided the focus of the microscope was the same in each case. On the contrary, the general distribution of the two structures appears to be quite different (compare fig. 3 with fig. 4). In figure 5, a photomicrograph, it is apparent that the Holmgren canals are not influenced by axone section, although the Golgi apparatus has undergone retispersion. The form of the Holmgren canal system differs from that of the Golgi apparatus. It is branching, often arranged concentrically. The canals are less tortuous, of greater diameter, and have a much more regular outline. The larger lacunae of the Golgi apparatus, joined by smaller tortuous threads, are never seen in the trophosphonium system. The canals at times communicate with the pericellular space, but the Golgi apparatus never.

Therefore the form and distribution of the two structures in the same neurone are frequently entirely different. Also, they do not react to axone section in the same manner. The conclusion is evident that the Golgi apparatus and Holmgren trophosphonium are not identical.

But what is the relationship of the two systems? This may be more easily studied in cells showing both simultaneously. Figure 6 is from a spinal ganglion of a cat seven days after section of the sciatic on that side. The Golgi apparatus, which appears to be undergoing retispersion and also retisolution, has been impregnated with silver by the Cajal method and the Holmgren canals

are simultaneously demonstrated by polychrome-methylene-blue. Nissl bodies are almost entirely absent from the cell except just about the nucleus. When the drawing was made the nucleolus was kept constantly in focus. The section of the cell drawn has therefore no greater thickness than that of the nucleolus. The

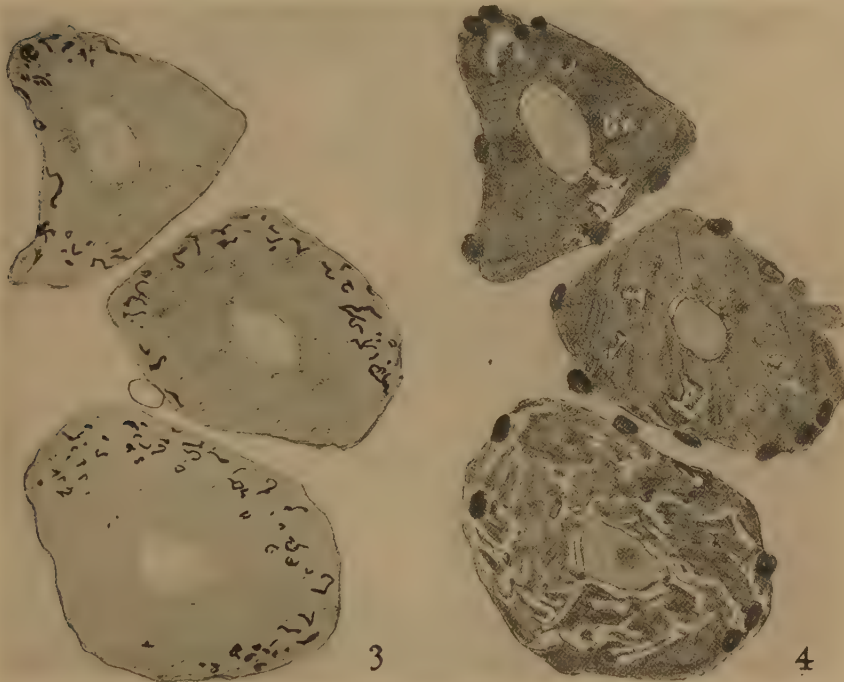
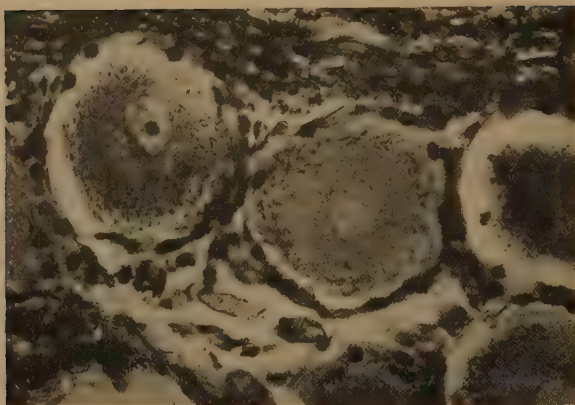


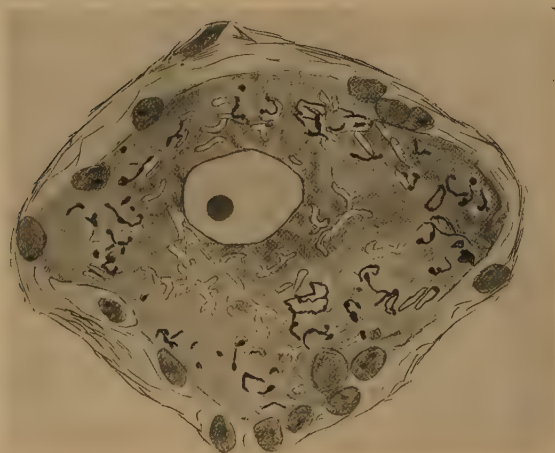
Fig. 3 Group of three cells from spinal ganglion of cat seventeen days after section of the posterior nerve roots. Retispersion and retisolution evident. Cajal silver preparation. Object. homog. immers. 1.5 mm. eye-piece no. 1.

Fig. 4 Same cells and magnification as in figure 3. The silver has been removed and followed by iron hematoxylin. In this drawing, as in figure 3 also, the nucleus at its greatest width was kept in focus while each cell was being drawn.

same contrast in the general location and form of the two structures as was noted above is evident. But in some areas a part of the Golgi apparatus is seen to lie against the wall of a Holmgren canal and to follow its contour. Subsequent staining of the same cell with iron hematoxylin showed the same canals. It is, no



5



6

Fig. 5 Photomicrograph. Magnification of 900, Cajal silver impregnation with polychrome-methylene-blue counterstain. Cells of spinal ganglion of cat subsequent to axone section. Golgi apparatus dark. Holmgren canals unstained. In the less darkly shaded cell the Golgi apparatus is at the periphery in evident retispersion, while in the center of the cell may be seen Holmgren canals, faintly outlined in two concentric groups. The chromophil substance has largely disappeared from this cell. In the darker cell the presence of the blue-stained chromophil substance partially obscures the black Golgi apparatus at the periphery of the cell, but demonstrates the Holmgren canals both in the center and at the periphery with greater distinctness.

Fig. 6 Cell from spinal ganglion (fig. 2). Simultaneous staining method. Same magnification as figure 1.

doubt, such appearances which have led Holmgren to speak of canalization of the trophospongium. The part impregnated with silver in this section would, I presume, according to his interpretation, be that portion of the trophospongium wall not liquefied. Figure 7, a photomicrograph of normal cells, shows these two systems clearly and it is apparent that the Golgi apparatus frequently lies beside the unstained canals.

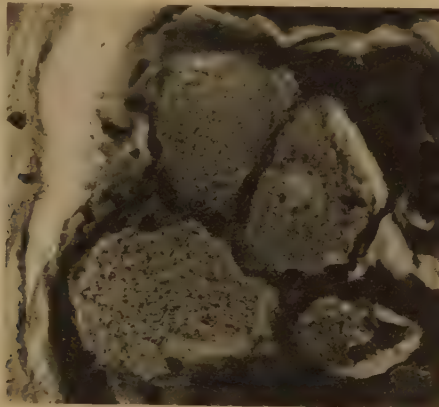


Fig. 7 Photomicrograph. Magnification of 900. Treated as in the successive staining method without, or with very little, removal of the silver from the Golgi apparatus. Cells of normal spinal ganglion of cat. In the lower large cell the relation of the Golgi apparatus, in black, to the Holmgren canals, unstained, is illustrated.

If one were to adopt the theory of von Bergen, on the other hand, that these canals are really artifacts or cracks in the cytoplasm, it would be sufficient to say that the crack tends to occur along the outline of the reticulum in certain cases. Subsequent staining of this cell with iron hematoxylin showed the same canals. In view of the excellent work of Holmgren and also the regular consistent appearance of the canals when the tissue is fixed without the slightest trace of shrinkage or distortion, it would seem extremely unlikely that these Holmgren canals are artifacts in any sense.

The above facts show that the Holmgren canals are not simply the negative picture of the Golgi apparatus. Fixation has been

in formol-uranium-nitrate solution which does not dissolve the Golgi apparatus. It is conceivable that if Carnoy's fluid, for example, were used for fixative, that the Golgi apparatus, after being dissolved or changed in some fashion, would then leave its negative image in the form of unstained canals. It does not seem likely, however, that these negatives would bear much resemblance to the canals of Holmgren. All reason for the negative-picture hypothesis disappears when the two structures are demonstrated simultaneously in the same normal cell (fig. 7) and are shown to react differently to abnormal conditions.

Occasionally, as noted above, I have seen canals and Golgi threads lying side by side. This may indicate, perhaps, a close association of function, but, inasmuch as we do not understand the function of either structure, the nature of their interrelation can only be conjectured.

SUMMARY

A short review has been made of the work which has been done, chiefly in continental laboratories, on the Golgi apparatus. It has been described as a cytoplasmic constituent of the cells of all animal organisms. It has the power of growth and has been followed through cell development and division. Its form is generally that of a network, but in certain of the lower invertebrates it appears as a thread or rod.

The trophosphonium-canal system described in the cytoplasm of certain cells by Holmgren has long been urged as identical with the Golgi apparatus. Both systems find their most complex development in the cytoplasm of nerve cells.

Cajal, Duesberg, Bensley, Cowdry, Nusbaum, Weigl, and others have refused to accept the extracellular connections described by Holmgren. They have maintained or implied that the Golgi apparatus and Holmgren trophosphonium were the same structure in nerve cells; that this structure appeared as a network which may be impregnated with silver or with osmic acid only after adequate fixation, and that, in case the fixative was inadequate or contained certain solvents, the structure appeared as an unstained canalicular system.

Golgi and his pupils, Kopsch, Bethe, and others, have never accepted the identity of the Golgi apparatus and Holmgren trophospongium, because of the difference in their form and also the extracellular connections of the canals described by Holmgren. On the contrary, those just mentioned above, who take a similar view with Cajal, find the form of the two structures similar and deny extracellular connections. Their general location within the normal cell is admitted to be the same.

The confusion seems to be due partly to the variety of methods of study, but also to the fact that comparison has been made in cells of the same type, but not the same cell, and there has been no means of inducing a specific change in either structure. It has been shown above that after the Golgi apparatus, as demonstrated by the Cajal method, has been studied in a certain cell, the silver may be removed and the trophospongium demonstrated in the same cell by iron hematoxylin; also that the two structures may be demonstrated simultaneously, as illustrated in the drawings and photomicrographs.

These methods provide a simple means of direct study and comparison. In the successive staining method no canals are found which correspond to the Golgi apparatus previously demonstrated. During retispersion (a specific alteration of the Golgi apparatus described in a previous publication) (39), the Golgi net is displaced to the cell periphery, while the intracellular location of the Holmgren canals is not altered.

CONCLUSIONS

That the Golgi apparatus is a universal cytoplasmic constituent has been established by a large number of investigators. In neurones the apparatus reacts to axone section in a specific manner. There is no similar response on the part of Holmgren's trophospongium. The two structures may be demonstrated independently in the cytoplasm of the same neurone, either successively or simultaneously.

The conclusion is evident. There cannot be a positive and a negative picture of the same structure, as has been widely maintained. They are separate structures demonstrable simultaneously.

Occasionally there appears a close anatomical relationship between parts of the Golgi apparatus and Holmgren's trophospongium which may indicate an intimate association of function. The methods described above provide a simple means of comparative study.

Further work upon the Holmgren canals is required to clearly demonstrate the developmental stages and, in fact, their existence antemortem.

In conclusion it is a pleasure to thank Professor Sherrington for his interest and suggestions. To Prof. Arthur Thomson I am indebted for helpful criticism, and to Mr. Chesterman, of the Anatomical Department, for the photomicrographs.

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Resumen por el autor, Paul H. Stevenson.

Sobre una anomalía poco común del *peroneus tertius* de un chino.

Una interesante anomalía del músculo *peroneus tertius* descubierta en las dos piernas de un chino constituye la base del presente trabajo. La anomalía consiste en la presencia de un *peroneus tertius* muy desarrollado, que se origina prácticamente en toda la extensión de la parte anterior de la superficie media del peroné. La inserción de este músculo tan desarrollado es próximamente la misma que la del músculo normal. El autor menciona brevemente la interpretación comun mente aceptada, que considera a este músculo "esencialmente humano" como una porción diferenciada del extensor largo de los dedos, y la consiguiente interpretación de las variaciones ordinarias de este músculo como simples variaciones en el grado de esta diferenciación. La anomalía discutida por el autor parece más bien representar una usurpación casi completa de la posición del extensor largo de los dedos, sin una correspondiente asimilación de la función de este último músculo. La presencia de una interesante deformidad en el calcáneo del pié derecho del mismo cuerpo se describe tambien en el presente trabajo. El autor plantea la cuestion de si el desarrollo exagerado del *peroneus tertius* y la tendencia aumentada resultante hacia la fuerte eversión del pié pueden o no considerarse como un factor en la producción de la deformidad descrita.

Translation by José F. Nonidez
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ON AN UNUSUAL ANOMALY OF THE PERONEUS TERTIUS IN A CHINESE

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ONE FIGURE

The peroneus tertius has long been recognized as a muscle appearing only in the human body,^{2, 4} and as such naturally attracts to itself a peculiar interest. It is usually considered to be a differentiated portion of the extensor digitorum longus,¹ and its variations therefore, which are very frequent, are commonly interpreted as mere variations in the degree of differentiation from this muscle.^{1, 2, 3, 7} The anomaly herewith presented however, seems to represent more than a simple variation in the degree of differentiation of one muscle from another, and differs sufficiently from those already described to make a note of it seem desirable.

The muscle in question was observed in a Chinese male, age 54 years, during the routine dissection of the lower extremity by first-year medical students. In both legs the muscle arose by a fleshy origin from the entire length of the anterior part of the medial surface of the fibula, with the exception of about 8 cm. at the extreme proximal end of the bone. The proximal 5 cm. of this latter space was occupied in turn by the diminutive origin of a very small extensor digitorum longus. The lower margin of the latter muscle and the upper margin of the anomalous peroneus tertius were both well rounded and distinctly formed, and separated by a distinct gap of approximately 3 cm. The peroneus tertius gave rise to a strong tendon which passed as a single structure beneath the transverse and cruciate ligaments of the ankle and divided into two separate tendons which became inserted into the base of the fifth metatarsal and the first phalanx of the fifth toe, respectively. The

small extensor digitorum longus gave rise to a very slender tendon which began at the upper margin of the peroneus tertius and passed down the leg and under the ligaments of the front of the ankle as a separate structure, subdividing on the dorsum



Fig. 1 Semidiagrammatic drawing, showing relations of anomalous peroneus tertius and extensor digitorum longus. Other anterior leg muscles represented by their cut tendons only.

of the foot into three smaller tendons having their insertions on the proximal phalanges of the fourth, third, and second toes, respectively (fig. 1). The nerve supply to both muscles was from branches of the deep peroneal nerve.

The general picture of this anomalous peroneus tertius did not suggest a mere variation in the degree of differentiation from the extensor digitorum longus. It implied, rather, that the peroneus tertius had almost completely usurped the position of latter muscle without assuming a proportionate share of its function. The question as to what effect this condition would have on the movements of the foot immediately presented itself.

It is interesting to note that the calcaneus in the right foot of this particular cadaver showed an unusual deformity. That portion of the bone bearing the main articular facet and receiving the greater part of the weight of the body through the talus, was not only depressed as if impacted into the cancellous portion of the bone beneath, but was tipped laterally on its anteroposterior axis, indicating that the force responsible for this impaction came while the foot was in a position of strong eversion.

Keith⁵ and Wood Jones⁶ have both emphasized the evolutionary importance of the peroneus tertius as providing the finishing touch in the eversion of the human foot. It might be considered an interesting question as to whether or not the development of this muscle beyond a certain point would be a factor in the production of injuries of the type found in this body.

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Resumen por el autor, Paul H. Stevenson.

El conducto biliar extra-hepático del camello.

El conducto biliar extra-hepático del camello (*Camelus bactrianus*) no presenta en su tamaño o forma indicio alguno de compensación por la falta de vejiga biliar de esta especie animal. En términos relativos, puede decirse que el conducto es estrecho y largo. Entra en el duodeno relativamente más lejos de esta parte que en el caso de las otras especies estudiadas en las cuales no existe vejiga biliar. El conducto pancreático se reúne con el biliar en el último tercio de su recorrido y a veces presenta un doble orificio de comunicación con el conducto biliar. El examen microscópico de la musculatura del extremo inferior del conducto ha demostrado la existencia de un aumento de las fibras musculares circulares que rodean al extremo duodenal del conducto biliar, indicando de modo muy evidente la presencia de un esfínter definido en este sitio. El autor ha llevado también a cabo observaciones sobre los sistemas extrahepáticos del caballo, ciervo, y rata albina, especies que carecen normalmente de vejiga biliar, y los resultados obtenidos se han reunido en un cuadro y también en una lámina de diagramas dibujados a escala con el propósito de que puedan ser comparados.

Translation by José F. Nonidez
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THE EXTRAHEPATIC BILIARY TRACT OF THE CAMEL

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TWO TEXT FIGURES AND ONE PLATE (FIGURES THREE TO SIX)

Judd,¹ in an experimental study of the effect of the removal of the gall-bladder in dogs, cats, and goats, observed that all the extrahepatic ducts dilated after cholecystectomy. Mann,² in a series of investigations on a larger number of animals, including some in which the gall-bladder is normally absent, endeavored to ascertain the relationship between the size and capacity of the extrahepatic ducts of animals in which the gall-bladder is normally absent to that of animals normally possessing this structure. He found no compensatory enlargement of the extrahepatic tracts of the horse, deer, pocket gopher, and the white rat.

The object of the present work is, 1) to corroborate the observations of Mann on the horse, deer, and rat, and, 2) to add to the literature on the subject of the extrahepatic biliary system of animals without a gall-bladder by a description of that of the camel. Inasmuch as the observations on the horse, deer, and the rat are entirely in accord with those published in the recent literature,² their mention in this paper will be confined to inclusion in the table of measurements and the plate of diagrams in which the biliary tracts of the various animals have been reduced to a common scale for the purpose of comparison of form and size.

The material for this study of the camel (*Camelus bactrianus*) consisted of the entire abdominal viscera of two camels, an adult and her calf of five months. Since a camel attains full growth at sixteen years,⁶ the small animal represents a stage of growth comparable to an eight months' human infant.

The data desired for the purpose of this work consisted of the following items: 1) the general relations of the biliary tract to the surrounding structures; 2) the length of the tract from the liver to the duodenum; 3) the diameter of the duct at several points along its course; 4) the distance from the pylorus to the opening of the common bile duct in the duodenum and, 5) the relation of the bile duct to the duct of the pancreas.

The distribution and arrangement of the muscle fibers, especially in the region of the orifice opening into the duodenum, were studied by microscopic methods.

DESCRIPTION

In general, the biliary tract, as well as the rest of the abdominal viscera of the camel, with the notable exception of the stomach and caecum which are of typical ruminant character, resembles that of the horse more closely than that of any other animal. The duct arises at the hilus of the liver, on the concave visceral surface, by the junction of several large intrahepatic bile ducts. It almost immediately enters that portion of the gastrohepatic omentum which embraces the upper flexure of the duodenum (ligamentum hepatoduodenale). In this mass of connective tissue the duct lies ventral to and in close connection with the hepatic artery and the large portal vein. The first part of the duct in the case of the young camel was inclined to be tortuous, but was quite straight in the case of the adult animal. In the last half of its course the hepatic duct lies on the head of the pancreas and is joined by the short pancreatic duct in the manner described below.

The free portion of the biliary tract, from the liver to the duodenal wall, measured 11 cm. in the adult camel. Entering the duodenal wall at an angle of about 60° , the duct immediately changes its course within the wall to one parallel to the long axis of the duodenum. The portion of the biliary tract within the wall of the duodenum, and spoken of in the table as the intramural portion, measured 3 cm. The entire length of the extrahepatic biliary tract in the adult camel was therefore 14 cm.

The opening into the duodenum took the form of a crescentic slit with the convexity directed downward, and the orifice was not easily discernible among the plicae curculares of the intestinal mucosa.

The diameter of the duct proved to be remarkably uniform throughout its course. A very slight increase in size after the entrance of the pancreatic duct was noted, but was not in the least suggestive of a sacculation or of a dilatation as seen in this region in the tracts of some animals.⁷ In the adult camel the diameter of the upper part of the tract measured 0.9 cm., while below the entrance of the pancreatic duct this diameter was increased to 1.1 cm.

From the measurements made within the lumen of the duodenum, the orifice of the bile duct was found to be 40 cm. from the pyloric ring of the stomach.

At a point 2.5 cm. distant from its junction with the duodenal wall, the biliary tract is joined by a single large duct from the pancreas, and that part of the tract distal to this point acts as the common duct for the passage of both the pancreatic juice and the bile into the duodenum. The pancreatic duct approaches the bile duct at an angle of about 70°, and in a direction contrary to that of the flow of bile.

Upon opening the bile duct opposite the entrance of the pancreatic duct, an interesting condition was found in the animal. The pancreatic orifices were two in number, although the free portion of the pancreatic duct was plainly single and showed no evidence of division before its juncture with the biliary tract. The two orifices opened one at either end of a slight oval depression. The upper margin of this depression formed a distinct flap, or valve, over the upper or smaller orifice but the lower and larger of the two orifices was entirely open. In the case of the lower orifice there was apparently no valve-like structure present, unless the lateral walls of the oval depression approximated each other in life, forming a functional valve which would permit the escape of the pancreatic juice and at the same time prevent the passage of bile in the opposite direction.

It was largely to restudy this particular structure that the dissection of the second camel was undertaken. In the case of the second animal the pancreatic duct approached the biliary tract at more nearly a right angle, though still quite distinctly against the flow of bile. Upon opening the bile duct, some difficulty was at first encountered in locating the opening of the pancreatic duct. The orifice in this case was single and opened upon the floor of an oval depression, the lateral walls of which were closely approximated and reinforced by a sphincter-like arrangement of the mucosa. The protective mechanism against the passage of bile into the pancreas apparently consisted almost entirely of this redundancy of the mucous membrane surrounding the pancreatic duct orifice. Microscopic examination of that portion of the pancreatic duct entering the bile duct, in the case of the small camel, showed a few scattered muscle bundles, most of which assumed a longitudinal direction. No arrangement of muscle fibers into a circular sphincter-like band was recognizable.

As a possible explanation of the double orifice of the pancreatic duct in the adult camel, the question of a fusion of the accessory with the main pancreatic duct is suggested (fig. 1, come, C, and D). There was no separate accessory pancreatic (ligament present in either of the camels dissected in this study. The tissue at there was, however, in both instances, a small contribution from the head of the pancreas joining the main pancreatic duct not far from its juncture with the bile duct, lends but not to the suggestion of fusion as depicted in the diagrams. The presence of Brunner's glands in the submucosa of the accessory tract below the entrance of the pancreatic duct indicates a close developmental relationship between this portion of the tract and the duodenum, lending further support to the suggestion of fusion between the two pancreatic ducts which open separately into the corresponding portion of the duodenum in other animals.

The mucous membrane of the upper part of the biliary tract was perfectly smooth. Below the entrance of the pancreatic duct, however, the mucous membrane was thrown into minute longitudinal ridges. These ridges increased in size and number

toward the duodenum and finally merged with the plicas of the intestinal mucous membrane. Microscopic examination of this lower part of the tract showed Brunner's glands in the submucosa, as mentioned above.

Complete microscopic examination of the wall of the entire tract with special reference to the musculature was made. Muscle fibers, though present, were few and widely scattered

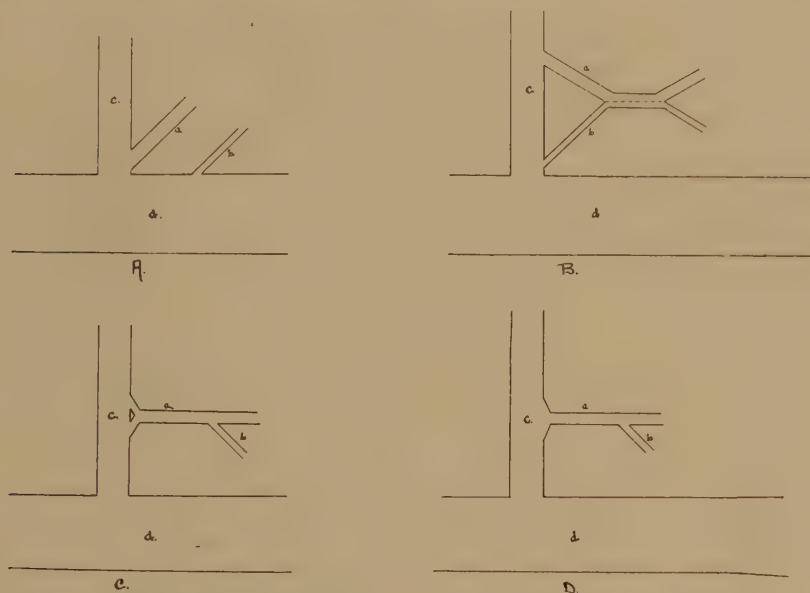


Fig. 1 Hypothetical development of the pancreatic duct in the camel. A. Biliary and pancreatic ducts as seen in the horse. *a*, main pancreatic duct; *b*, accessory pancreatic duct; *c*, biliary tract; *d*, duodenum. B. Theoretical stage. C. Biliary and pancreatic ducts as seen in camel no. 1. D. Biliary and pancreatic ducts as seen in camel no. 2.

in the upper part of the tract. They began to appear in distinct bundles, however, just above the entrance of the pancreatic duct. The direction followed by these bundles was in the main circular, but longitudinal and diagonal fibers were also present. In the wall of the duodenum there was a certain amount of blending of the muscle fibers of the bile tract with those of the intestine. This blending affected chiefly the longitudinal fibers.



Fig. 2 The extrahepatic biliary tract of the camel

The circular fibers, which became increasingly prominent as the bile-duct orifice in the duodenum was approached, remained distinct and assumed a sphincter-like arrangement around the terminal portion of the duct.

The question of a sphincter around the end of the bile duct has long been an interesting and a somewhat debatable one. Comparative studies of the structure in various species have been made by Oddi,⁹ Hendrickson,¹⁰ Mann,³ and others. Oddi reported the absence of such a sphincter in the horse, and the subsequent inference of many was that animals lacking gall-bladders were also without this sphincter muscle at the end of the bile duct. Mann's investigation was undertaken to ascertain the presence or absence of this sphincter in animals without gall-bladders. In four species of animals without gall-bladders (the horse, deer, rat, and pocket gopher) he found "a definite arrangement of muscle fibers which might functionate as a sphincter in each species studied" (l.c., p. 360). Comparing these findings with those on a series of ten animals having gall-bladders, he found that "no difference could be observed in animals that have a gall-bladder when compared with those without one. There can be no doubt that anatomically some species of animals lacking a gall-bladder have an arrangement of muscle around the duodenal end of the bile duct which can act as a sphincter" (l.c., p. 360).

The present study shows that there is a similar arrangement of the circular muscle fibers around the duodenal end of the biliary tract of the camel to form a sphincter.

SUMMARY

The camel (*C. bactrianus*) belongs to that group of animals in which the gall-bladder is normally absent. The extrahepatic biliary system of the camel shows no suggestion either in size or shape of compensation for the lack of the gall-bladder. Expressed in relative terms, the extrahepatic biliary tract of the camel is long and narrow. It enters the duodenum relatively much farther from the pylorus than in the case of any other species thus far studied in which the gall-bladder is absent. The

pancreatic duct joins the bile duct in the last third of its course, and sometimes presents a double orifice. The circular muscle fibers become augmented in the intramural portion of the tract and are formed into a distinct sphincter-like arrangement surrounding the duodenal orifice of the duct.

Table of measurements

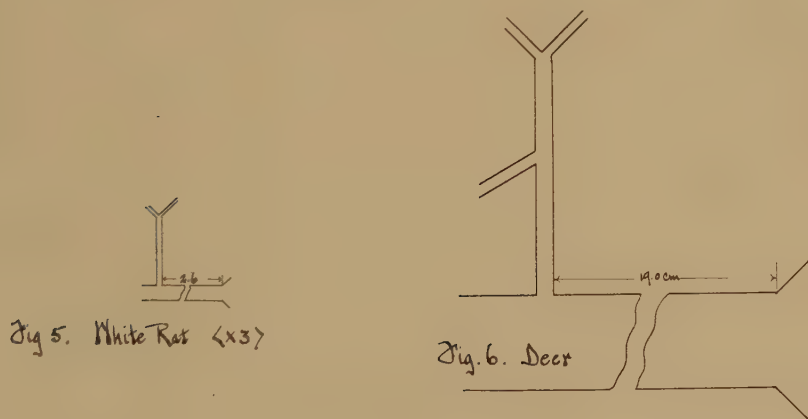
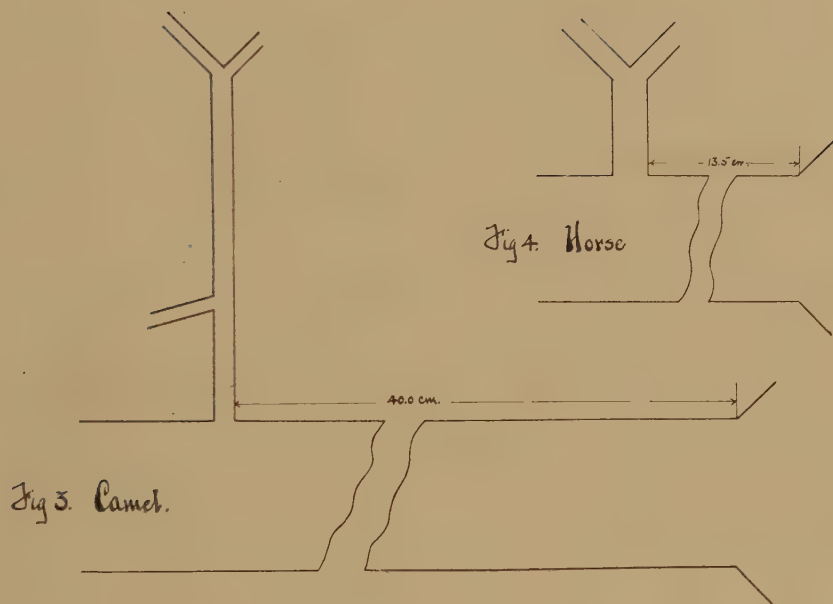
ANIMAL	LENGTH OF FREE PORTION	INTRA- MURAL PORTION	TOTAL LENGTH	DIAMETER	DISTANCE B-DUCT FROM PYLORUS
	CM.	CM.	CM.	CM.	CM.
Camel					
No. 1.....	11.0	3.0	14.0	0.7	40.0
No. 2.....	7.2	2.4	9.6	0.4	29.0
Horse	4.0	0.2	4.2	1.1	13.5
Deer.....	5.0	4.2	9.2	0.6	19.0
Rat.....	2.4		2.4	0.1	2.6

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PLATE 1

Schematic representation of relative sizes. Diagrams drawn to scale and conventionalized to correspond to similar diagrams by Mann.



Resumen por el autor, Berlin Berthold Nicholson.

Descripción de un método para exhibir y conservar los cuadros murales anatómicos.

El presente trabajo se ocupa de la descripción de un método efectivo de exhibir y conservar los cuadros murales anatomicos de gran tamaño.

Translation by José F. Nonidez
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DESCRIPTION OF A METHOD FOR THE DISPLAY AND STORAGE OF ANATOMICAL CHARTS

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TWO FIGURES

The service of large colored charts in the teaching of anatomy is widely recognized. Often, however, the value of these teaching adjuncts is much impaired, because of an unsatisfactory method of storing and handling the charts. In order that they may be of greatest service to the student and instructor, the charts should always be readily accessible and conveniently arranged; otherwise, they will seldom be utilized by the average busy medical student.

The purpose of this paper is to describe a method of handling charts, which Dr. Wilmer Baker, now of Tulane University, and I devised, and which a three years' experience has proved to be highly satisfactory. The method consists of employing frames made from some light wood and swung by hinges in rows along the wall (fig. 1).

The structure of the frame can best be understood by referring to the detailed drawing (fig. 2). It is important to make the frame as light as possible and at the same time substantial and rigid. It must be thoroughly braced to prevent the outer end from sagging. It is likewise important to have the various parts firmly nailed or screwed together. Experience has shown that it is best to fasten the two front strips, which serve to hold the ends of the charts in place, with small screws; or better still, to pass the screws through a small metal plate the width of the wooden strip. This renders them less liable to split or to become pried off during the removal and replacement of charts. The frame should be the same length as the chart and about one-half of an inch taller than the chart itself.

The actual construction of the frame is relatively simple in the hands of any good carpenter. To simplify matters and for the sake of uniformity, it is best to carefully and accurately cut all of the parts in a miter-box at the outset. Then the putting together of the frames is readily and easily accomplished.



Fig. 1 Photograph showing the arrangement of the charts along the wall. It also shows the spacing between the charts and the manner in which they can be folded back. The taller frames are constructed in a manner similar to the longer ones, details of the latter being shown in figure 2. The first frame of each type shown in the tier has its front chart pulled out about 2 inches. (Charts here shown are made by Hoedt & Co., Philadelphia.)

Special care must be taken in cutting and nailing in the brace. It must be cut as long as possible and placed as shown in figure 2. It is important to thoroughly toe-nail it at both ends and on both sides, as well as to nail through the ends of the brace. Thoroughly seasoned lumber must be used if the frames are to stand up as they should.

The frames are best hung to 2 x 4 timbers bolted to the wall. These timbers should be so placed that the hinges will be as near as possible to the top and the bottom of the frame. A minimum stress is thus placed upon the frame and hinges. The frames must

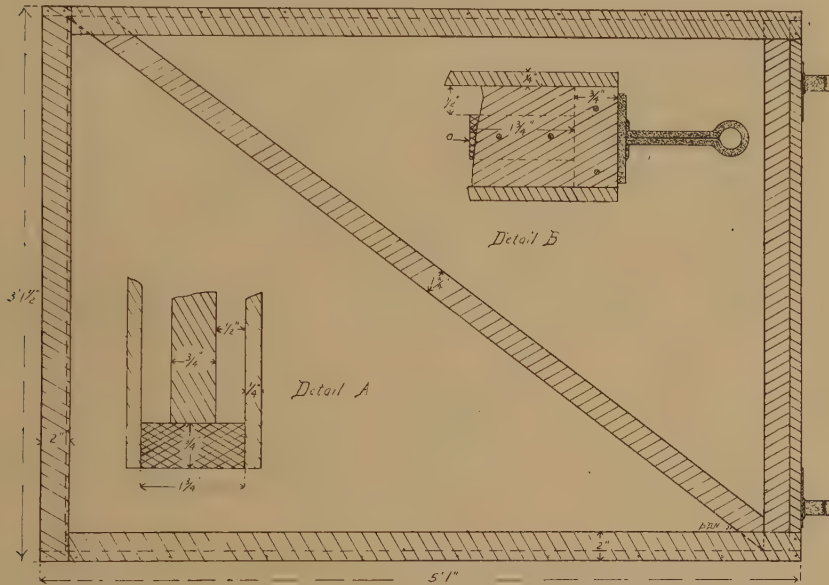


Fig. 2 Drawing showing side view of a frame. The broken lines show parts hidden from view. One half of hinge is shown, the other half bearing the post is fastened to the wall. Note the manner in which brace is placed. This position of brace offers the greatest possible strength.

Detail A. This shows the lower corner of the frame viewed from in front. Note the two $\frac{1}{2}$ -inch spaces for the charts, between which is a timber for the purpose of offering greater strength to the frame. The two lateral strips should be fastened with screws passed through a metal plate the length of the width of the strip.

Detail B. This shows the upper rear corner viewed from above. Note timber (a) which serves to offer rigidity and strength to the back of the frame; note also the manner in which the various parts serve to fasten the corner together.

be so placed that when they are swung toward the wall, the hinges will not be split off through the striking of the frame against the back part of its neighbor.

It is essential to use a hinge that has a long shank and one that is made in two parts, so that the frames can readily be re-

moved from their supports on the wall. With such a hinge the whole frame can be removed to another position and another readily put in its place, thus reducing to a minimum the handling of the charts themselves. Furthermore, when certain charts have served their purpose in a particular study, they can readily be removed for storage in the stock-room.

The method here described pertains only to large stiff charts for use in the laboratory. For handling and storage of flexible wall charts for general use in the lecture-room and laboratory the reader is referred to the method recently described by Reighard (*Anat. Rec.*, vol. 19, pp. 39-46, 1920).

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Resumen por el autor, N. W. Ingalls.

Una cámara estereoscópica simple.

Esta cámara no es sino la cámara fotomicrográfica modelo H de Bausch & Lomb modificada para el trabajo estereoscópico. En breves palabras, los cambios introducidos consisten: 1. En un nuevo ajuste de la cámara y los soportes angulares que la mantienen en una posición vertical para traer la lente y eje óptico en coincidencia con el centro de rotación de dicha posición. 2. En la elevación del centro de rotación a una altura conveniente para permitir un espacioso campo de acción. Esto pudo conseguirse mediante el empleo de un pequeño trozo de barra I, cuya superficie superior se inclina 8 grados para compensar el hecho de que el instrumento original solo permite movimiento lateral a la perpendicular. De este modo se puede colocar un objeto en el centro de rotación, enfocándole con la cámara vertical y haciendo exposiciones con la cámara inclinada de 3 a 5 grados a cada lado de la perpendicular. La tuerca opinza de que está provisto el instrumento original servirá para mantener la cámara en la posición deseada. Los objetos planos pueden fotografiarse con mayor ángulo que los objetos que poseen considerable profundidad. El ángulo disminuye también con el aumento de la distancia del objeto. Un ángulo demasiado grande tiende a deformar la imagen al exagerar la profundidad.

Translation by José F. Nonidez
Cornell Medical College, New York

A SIMPLE STEREOSCOPIC CAMERA

N. WILLIAM INGALLS

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ONE FIGURE

The advantages of a stereoscopic photograph over the ordinary, single, flat photograph are so great and far-reaching that a brief account of the relatively simple apparatus necessary seems in order. For some little time the Anatomical Laboratory has been making extensive use of stereo-photographs, both as records and in routine teaching.

For purposes of record the method all but replaces the actual specimen, and for material which cannot for any reason be preserved, as embryological or histological material, the results are all that could be desired. The same applies also to objects, dissections, and the like, where, on account of their large size, mounting and preservation may not be practicable. The results with small specimens, such as one would ordinarily examine with the aid of a binocular, are especially gratifying when the photographs have been taken under a moderate magnification.

The use of stereophotographs as records of the human embryos in the department collection and also the methods of stereoprojection were demonstrated at the March meeting of the Anatomists in Philadelphia. The slides illustrating Doctor Todd's paper were all of bone specimens and ranged from reductions to enlargements; the slides of the author, representing embryological material, were mainly enlargements. The stereoprojection as shown at this meeting was the same as that in use in the department teaching. The same negatives furnish both lantern slides and prints, so that the student has the opportunity to review what he has seen on the screen by means of the mounted prints in the hand stereoscope.

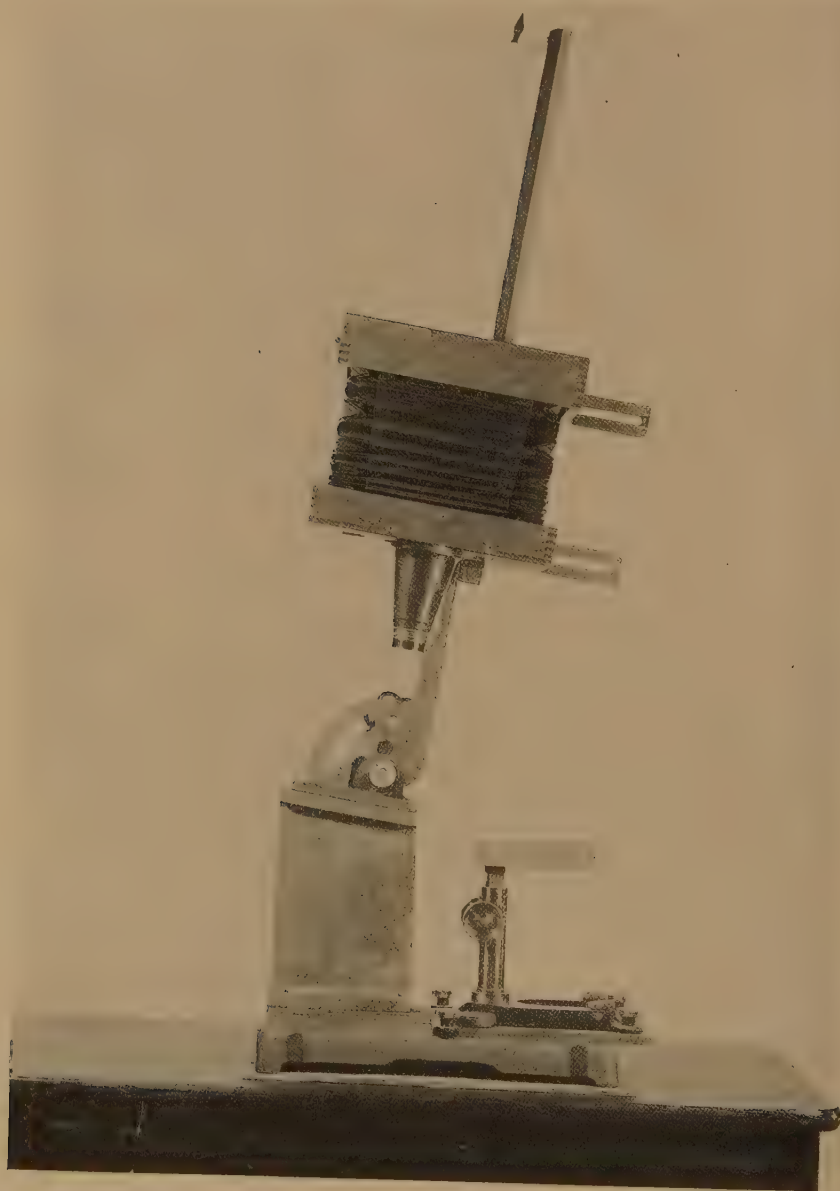


Figure 1

The stereocamera now in use is shown on the accompanying plate. This represents the Photomicrographic Camera Model H of Bausch & Lomb as modified by the author to meet the requirements of stereoscopic photography. Two alterations were necessary to adopt the camera to stereoscopic work. In the first place, the lens had to be brought over the center of rotation of the supporting arm, i. e., the optical axis of the camera must pass through the center of rotation. In its original form the camera box is placed on one side of the vertical support—on the right in the view represented—and some provision was made for lateral adjustment of the camera on this support. Since it was not possible to shift the lens over the center of rotation, it was necessary to remove the camera from the L-shaped brass straps to which it was attached, reverse these straps in the lateral supports, and replace the camera box. The pin with milled head which clamps the camera in lateral adjustments had to be removed and replaced in the brass strap nearer the angle, in order to allow the camera box to be pushed over the center of rotation. The manner in which the camera box has been readjusted may be seen from the original screw holes.

The second alteration serves a double purpose. It was necessary to raise the center of rotation to a sufficient distance above the base to provide working room, to accommodate objects of varying size and to permit their adjustment in a plane passing through the center of rotation. This was accomplished by removing the entire camera support from the flat base and placing it on top of a rather massive casting, I-shaped in section. It will be noticed that the top of this new support is higher at one end. The original mounting allows of movement only on one side of the perpendicular, so that by inclining the top of the new support the vertical axis of the instrument can be inclined about 10° on one side of the perpendicular and, with the old adjustment, nearly 90° in the opposite direction. The original clamp will hold the instrument in any desired position.

It is now possible to place an object in the center of rotation, which is in this case 230 mm. above the base, and to swing the camera to either side of the vertical about this same center.

Rotating the object instead of the camera has little if anything to recommend it, is obviously impracticable in most cases, and has the further disadvantage of changing the lights and shadows on the object.

The specimen to be stereoscoped is placed in the plane of the center of rotation and the camera focused as usual. Points exactly in the center will not change their position on the ground-glass when the camera is inclined to either side. Points at a higher or lower level or on the right or left of the axis of rotation will change their position. Since only a small linear portion of any object can lie in the axis, a very accurate adjustment is not called for. The small, remodeled dissecting microscope shown in the illustration is a convenient means of securing the important vertical adjustment of the object. To the upper end of the arm supporting the camera is attached a pointer which moves over a graduated arc on the wall close behind the instrument, so that the vertical position and the amount of inclination to either side can be readily determined. Much of our work has been done with an inclination of 5° or 6° on each side of the vertical, or a difference of 10° or 12° in the two positions of the camera. If the object is of considerable depth, a smaller total angle, 8° , 6° , or even 4° may be used, and the angle would naturally be reduced as the distance between the object and the lens increases. Too great an angle results in a distorted effect by exaggerating the depth.

The lens usually employed for objects of moderate size and also for enlargements is a 72-mm. tessar Ic. In the illustration this is mounted on an aluminum adapter 75-mm. long, to allow better illumination of the field. For large objects and with the camera at a greater distance, a 5 x 7 protar, series V, has given very satisfactory results.

Two exposures are made, on $3\frac{1}{4} \times 4\frac{1}{4}$ plates, at an equal inclination on either side of the vertical, 4° , 5° , or 6° if the total angle is to be 8° , 10° , or 12° . If a better view of the object can be obtained by inclining the camera, this altered position may be taken as the starting-point and the exposures made a given number of degrees to each side of this point. This is very convenient

in securing accurate views of objects which it may not be easy to adjust as a dorsal or ventral view of a small embryo. The finished prints are mounted as the negatives were taken, the right-hand view on the right and the left on the left and in such relation to each other that, as seen through the stereoscope, the two views fuse easily and completely. This is accomplished best by fixing one in place temporarily and making the adjustment with the other print while viewing both through the stereoscope. If the prints are reversed, the left on the right, the result is a reversal of the perspective, an intaglio instead of cameo, or vice versa.

The limitations of this instrument are due to the fact that the optical axis is only about 10 cm. in front of the vertical support. Consequently objects over 20 cm., 8 inches long, cannot be centered unless placed crosswise in the field.

Resumen por los autores, Waro Nakahara y James B. Murphy.

Sobre la naturaleza del llamado centro germinal en el tejido linfoide.

En una serie de estudios sobre la estimulación linfoide inducida artificialmente en varios animals han hallado los autores un marcado aumento en el número de figuras mitósicas en los centros germinales linfoides, el cual tiene lugar antes de la manifestación de la linfocitosis en la sangre, sin depender del método empleado para producir esta condición. Este paralelismo ofrece un apoyo experimental a la teoría original de Flemming, indicando la naturaleza linfoblástica de los llamados centros germinales de los órganos linfoides. Los métodos empleados para producir la estimulación linfoide fueron: 1) Inyección de tejido vivo homólogo seguido de inoculación de un tumor; 2) Exposición a una temperatura seca; 3) Exposición a una pequeña dosis de los rayos X, y 4). Exposición a una pequeña dosis de los rayos X seguida de inoculación de un tumor.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE NATURE OF THE SO-CALLED GERM CENTER IN LYMPHOID TISSUE

WARO NAKAHARA AND JAMES B. MURPHY

The Rockefeller Institute for Medical Research

TWO FIGURES

INTRODUCTION

The lymphopoietic function of the spleen and lymph-nodes is a well-known fact, but as to just how the production of new lymphocytes is brought about in these organs is still a matter of uncertainty. Flemming ('85) first called attention to the frequent occurrence of mitosis in the tissue of lymphoid organs, especially at a certain spot in the follicle. To this spot he gave the name 'germ center,' believing it to be a birth-place of new lymphocytes. The view has been traditionally followed in numerous texts, in which the lymphoblastic nature of the cells of the germ center is generally accepted and is based entirely on the interpretation of the static 'picture' as observed in preserved material. On account of the obvious limitations of this method of observation, the deductions made on it lack finality.

In the course of experiments on various types of general lymphoid reaction, we have collected observations which may serve to throw additional light on the process. A résumé of the observations and a brief discussion bearing on them are given in this paper in the hope of elucidating somewhat the point under consideration.

OBSERVATION

Lymphoid reaction induced by dry heat

It was reported by Murphy and Sturm ('19) that animals subjected to a small exposure of dry heat (using an electric heating lamp as the source of heat) show, following a sharp initial fall, a

rise of circulating lymphocytes often amounting to more than 200 to 300 per cent above the normal. Polymorphonuclear leucocytes participate in the fall, but they recover their normal level slowly and never rise above it.

Histological examination (Nakahara, '19) of the spleen and lymph-nodes of animals carried as a parallel to the above experiment showed that these organs, immediately after the heat treatment, contain numerous necrotic cells. The dead cells were present abundantly in the nodules and in the pulp of spleen, cortex, and lymph-cords of nodes. The cells of the germ centers were apparently unaffected, but cell division was totally suppressed.

It was also observed at about forty-eight hours after the treatment that, along with an elimination of the necrotic cells, there was an abnormally large number of mitotic figures present in the germ centers, and this hyperactivity of the centers continued for several days.

Lymphoid reaction in immunity to transplanted cancer induced by an injection of homologous living tissue

Mice, as is well known, can be made relatively resistant to transplanted cancer by injecting them with an emulsion of homologous living tissue ten days before inoculating the cancer grafts. Murphy and Morton ('15) found that the lymphocytic elements of the blood showed an active increase during this process, while polymorphonuclear cells were not appreciably affected. No change in the lymphocytes occurred in the resistant mice until the cancer graft was introduced, when an immediate and sharp rise took place in these cells. In the majority of animals the increase was 100 to 200 per cent above the normal and endured about two weeks.

The histological study (Murphy and Nakahara, '20) made of preserved material from the inoculated and resistant mice showed in the germ centers of the lymphoid organs increase in mitotic figures, reaching a maximum about five days after the immunizing injection and followed by a fall, the normal being regained by the tenth day. The resistant mice when inoculated with a cancer

graft ten days after the tissue injection show a second great increase in the mitotic figures, particularly of the germ centers. Judging from the number of mitosis, this second stimulation is more intense than the first.

Lymphoid reaction induced by small doses of x-rays

The selective action of X-rays on the lymphoid tissue has been recognized since the work of Heineke ('05). It was shown in this laboratory that while large doses of x-ray are destructive to, small doses bring about an increase in the lymphoid cells (Murphy and Morton, '15). In the case of rabbits thus treated, the number of circulating lymphocytes rose often in the course of one week (Thomas, Taylor, and Witherbee, '19) 100 per cent above the normal.

Histologically (Nakahara, '19) in these animals no appreciable destruction of cellular elements of spleen and lymph-nodes was observed; nevertheless the mitotic figures increased gradually in the germ centers, the height being reached in a few days after the treatment, and the increase persisted for a period of about two weeks.

Lymphoid reaction in immunity to transplanted cancer induced by small dose of x-rays in mice

Further, it was noted that in mice a certain small dose of x-rays of low penetration induces a stimulation of the lymphoid tissue (Nakahara and Murphy '20) and a regular and considerable increase in the number of mitotic figures of the germ centers takes place from twenty-four hours to four days after the treatment.

When cancer grafts were inoculated in these mice seven days after the x-ray treatment, an appreciable increase in resistance as compared to the normal untreated mice was detected (Nakahara and Murphy, '21). The blood picture of the mice, thus rendered comparatively more resistant, showed no constant change following the x-ray treatment, but the lymphocytes were found to rise 100 per cent or more above the normal within two weeks after can-

cer-graft inoculation (Nakahara and Murphy, '21). As in other varieties of cancer immunity, the polymorphonuclear leucocytes showed no appreciable change.

There was some variation observed in the histological appearance of the lymphoid organs of mice first x-rayed and inoculated with cancer grafts seven days afterward, but as a rule a profound acceleration of the rate of cell division was present in the lymphoid tissue, and especially in the area of germ centers (Nakahara and Murphy, '21).

DISCUSSION

The material which supplied the above data was studied primarily from the point of view of our interest in the relationship between the lymphoid cells and resistance to transplanted cancer in mice. In cancer immunity, induced by the usual means, namely, by the injection of living homologous tissue, it was noted that a marked increase in the circulating lymphocytes occurs, with which is associated an increase of cell multiplication by mitosis in the germ centers of the lymphoid organs. This relationship between the resistance to cancer inoculation and lymphocytosis was observed, regardless of means by which the lymphocytosis is induced. The rise of the lymphocytes of the blood is invariably preceded by an increased proliferative cellular activity in the germ centers. These facts suggest that lymphoid germ centers are really made up of lymphoblastic tissue, and are among the sources of lymphocytes. The cells comprising the so-called germ centers and the lymphocytes making up the nodule of lymphoid organs are often stated to differ morphologically, but in reality there is no criterion which serves actually to distinguish one from the other.

The fact that phagocytosis sometimes occurs in the area of germ centers and is absent from the lymph-nodules may be urged as evidence that the cells of the germ center are not lymphoid in nature, as lymphocytes are non-phagocytic. Such a deduction would seem too dogmatic, since embryonic mesenchymal cells, from which lymphoid tissue develops, are admittedly phagocytic. Moreover, it would be difficult to decide whether the large cells containing ingested cell fragments (Flemming's 'stainable particles')

really originate from the cells indigenous to germ centers or come from without. It is well known, also, that new lymphocytes are produced by a division of a preexisting one, which is capable of mitosis. This obviously cannot be considered as the only possible method of lymphocytic proliferation, as, embryologically, it is conceivable that cells with the potentiality of developing into lymphocytes may coexist in the adult along with the already differentiated lymphocytes.

SUMMARY

Several kinds of lymphoid reactions are described in which stimulation of the cell division of the germ centers of lymphoid organs preceded lymphocytosis in the blood. The association of the two processes described provides evidence that the lymphoid germ centers are in fact sources of blood lymphocytes, as the name indicates.

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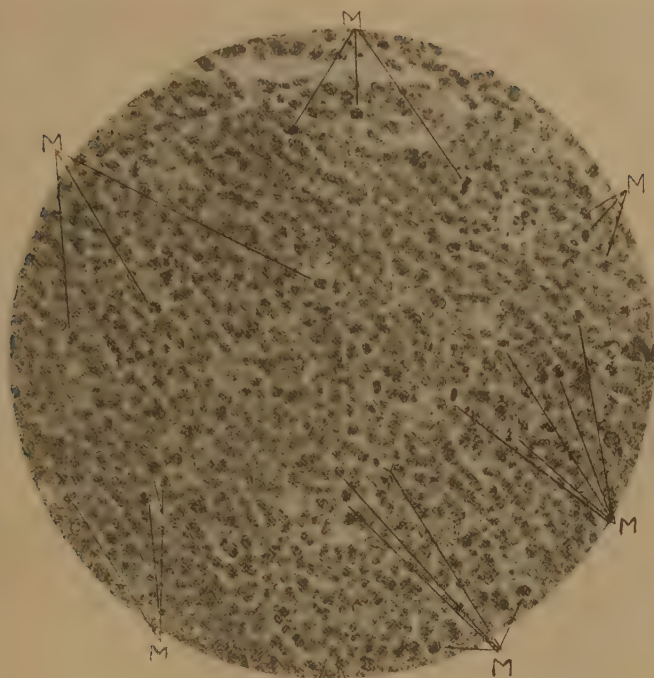
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PLATE 1

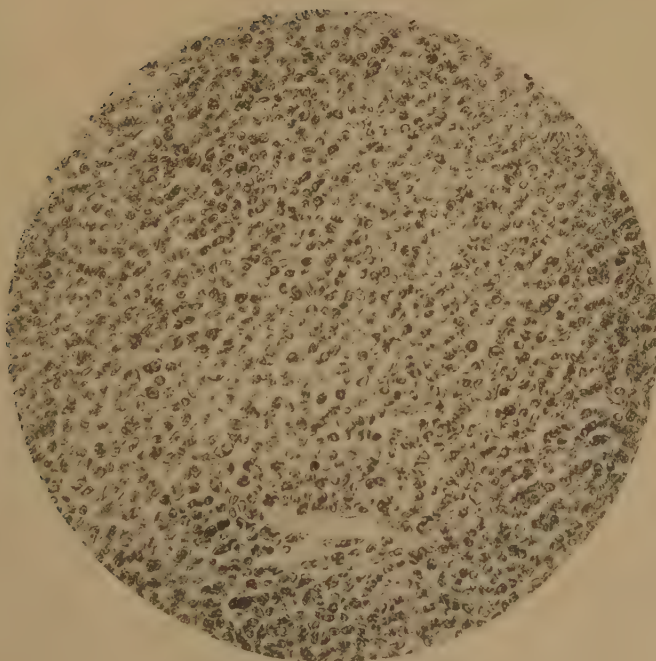
EXPLANATION OF FIGURES

1 A germ center of lymph-node of mouse treated with a small dose of x-rays of low penetration. Note the hyperactivity in cell proliferation as indicated by an unusually large number of mitotic figures (*M*). The majority of mice thus treated are potentially more resistant to transplanted cancer than untreated mice, and show, upon cancer inoculation, a marked increase in the number of circulating lymphocytes. $\times 480$.

2 An inactive germ center of lymph-node of mouse. Note the absence of mitotic figures. $\times 480$.



1



2

Resumen por el autor, C. D. Fife.

Ausencia de la carótida común.

En todas las formas esta condición es muy rara, debiéndose a una interrupción en el tercer arco aórtico y a la persistencia de la raíz aórtica dorsal entre los arcos tercero y cuarto. Este parte de la raíz dorsal aórtica es el conducto de Botal, que constituye un vaso funcional en muchos reptiles, especialmente manifiesto en *Sphenodon*. En el caso estudiado por el autor, el de un varón negro, falta la carótida común derecha, existiendo además un arco aórtico derecho; y la subclavia izquierda es del tipo normal arqueado, estando formada su porción proximal por la aorta dorsal izquierda más allá del cuarto arco, la cual recibe el conducto arterial. La disposición particular de las ramas del arco de la aorta es la consecuencia de la retención del cuarto arco derecho en vez del izquierdo. La rareza de la anomalía de la carótida es una expresión de la gran constancia con que la aorta dorsal se interrumpe entre el tercer y cuarto arco. La interrupción es muy antigua, estando ya bien establecida en los reptiles. También existía en el caso estudiado una vena cava superior izquierda que carecía de relación alguna con la derecha.

Translation by José F. Nonidez
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ABSENCE OF THE COMMON CAROTID

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THREE TEXT FIGURES

Absence of the common carotid is one of the rarest of the many anomalies which take their origin in the varying transformations of the aortic arches. Combined, as it is here, with other cardiac anomalies, it places the heart in question in a rather unique position.

On account of the rarity of the conditions found here and also because of the embryological interest which attaches to them, I have undertaken at Doctor Ingall's suggestion a brief account of the arterial anomalies and of their developmental significance.

The specimen, T. 71 of the teratological collection, was found in a dissecting-room subject, body no. 662, a male negro between thirty-five and forty years of age. Upon opening the thorax the heart was found in approximately its normal position. The aorta was directed upward and somewhat to the right along the right side of the trachea and oesophagus, the summit of the arch being just below the level of the disc between the first and second thoracic vertebrae. It then descended very obliquely toward the left, reaching its normal position on the left side about the level of the seventh vertebra. Corresponding with the oblique course of the aorta across the vertebral column, there was noticed a faint depression on the column extending from the second to the seventh vertebrae. Between the second and fifth vertebrae the oesophagus was inaccessible from the right side.

The branches of this right aortic arch are as follows (fig. 1): The first branch is the left common carotid which takes origin from ventromesial aspect of the first part of the arch close to the trachea and about 3 inches above the aortic ring. Next in order,

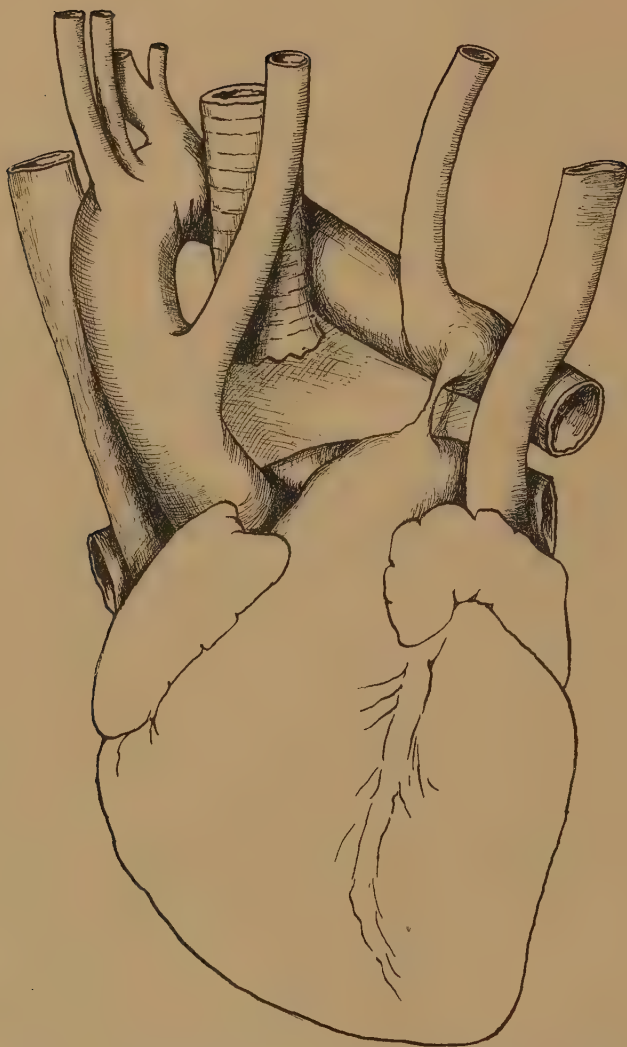


Fig. 1 Heart and great vessels, T. 71 of the teratological collection. The aorta and left cava have been displaced somewhat to show the arrangement of the vessels. Description in text.

and close together, near the summit of the arch, are the right external and internal carotids, the external almost immediately in front of the internal. While these two vessels are quite close together, their origins from the aorta are quite separate and distinct. The fourth branch is the right subclavian not far from the carotids, while the fifth and last vessel, the left subclavian, arises $2\frac{1}{2}$ inches beyond the right from the ventromesial wall of the last part of the arch. This last-mentioned branch of the aorta presents at its origin a definite bulbous enlargement such as is usually found in this type of left subclavian. This enlargement measures approximately $\frac{1}{2}$ inch in diameter and from its apex the ligamentum arteriosum passes to join the pulmonary artery at the point of bifurcation. A very similar arrangement of the branches of the arch may be noted in the case of double aortic arch of Leboucq as reproduced by Broman ('11, l.c., fig. 460).

The other major deviation from the normal in this heart was the presence of a left superior cava, which received the blood from the superior intercostal and hemiazygos superior of the same side.

It may be noted in passing that this subject presented other minor aberrant conditions: an hiatus in the posterior arch of the atlas, a perforation of the sternum at the level of the fourth interspace, a supratrochlear foramen in each humerus, and a lateral internal mammary artery on the left side.

The interesting features in the specimen under discussion are as follows: 1) The presence of a left superior cava; 2) the right aortic arch with the associated relations of the left subclavian artery and ligamentum arteriosum, and, 3) the absence of the right common carotid.

The first point does not call for any discussion at this time. The condition is not especially rare and in this case the usual arrangement obtains in which there is no anastomosis between the two superior cavae (McCotter, '16).

The arterial arrangement is readily explained as may be seen in the diagram reproduced in figure 3. Right aortic arches are not particularly rare, Abbot ('92), Reid ('14), and many others have reported similar conditions. Poynter ('16) distinguishes four groups under the heading of 'right aortic arch.' Our case

represents the most common variety, in which there is an obliteration of the left fourth arch and of the dorsal roots beyond, with a persistence of the left dorsal aorta. The developmental history of these cases, as shown in figure 3, makes clear the low origin of the left subclavian from the descending aorta as well as

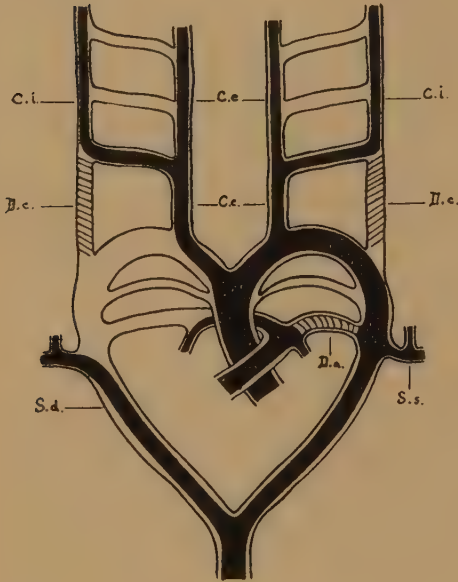


Figure 2

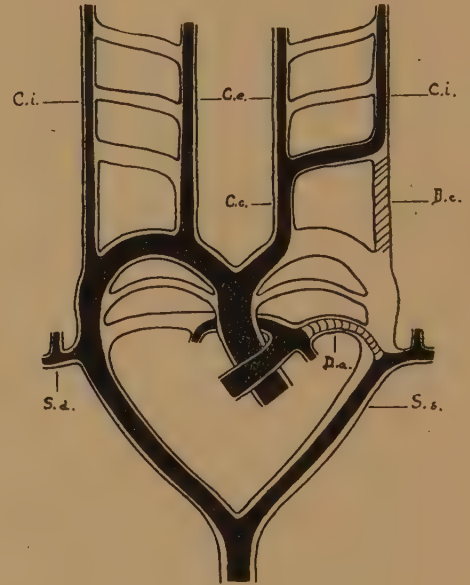


Figure 3

Fig. 2 Transformation of the aortic arches in those cases in which the right subclavian is the last branch of the arch.

Fig. 3 Present case, right arch with the usually associated type of left subclavian, absence of right common carotid.

C.c., carotis communis; *C.e.*, carotis externa; *C.i.*, carotis interna; *D.a.*, ductus arteriosus (ductus Botalli); *D.c.*, ductus caroticus; *S.d.*, subclavia dextra; *S.s.*, subclavia sinistra.

its relations to the ligamentum arteriosum. Almost constantly there is found a definite enlargement of the subclavian at its origin, and into this enlargement is inserted the remains of the ductus arteriosus. The ductus exhibits a remarkable constancy, it is rarely found on the right side even in cases of right arch.

At this point we would call attention to figure 2, which represents those relatively frequent cases in which the right subclavian is the last branch of the arch or arises from the descending aorta, and passes, in most cases, behind the oesophagus (cf. Holzapfel, '99; Banchi, '07). Associated with these atypical right subclavians, there is often a right thoracic duct. Except for the ductus Botalli, the more usual form of right aortic arch, and the right subclavian as the last branch of a left arch are mirror pictures of each other as may be seen from the accompanying diagrams. Both of these anomalous subclavians are in part dorsal aorta, but the commoner variety, on the right side, shows a much greater tendency to be shifted upward upon the aorta, closer to the left common carotid.

The carotids are of especial interest in the present case. As may be seen in the diagrams, the ventral aortic proximal to the fourth arches may give rise to a part of the aortic arch, a part of the common carotid or become the innominate. Although the carotids, the primitive and always the most important vascular channels to the head, may undergo extensive modifications and reductions, the absence of the common carotid constitutes one of the rarest arrangements of the branches of the aorta, and as an anomaly is very seldom encountered. Even a low bifurcation of the common carotid is extremely uncommon (Kantor, '05; Orr, '06). It is not necessary to explain the accompanying schematic diagrams, but we would direct attention to that portion of the dorsal aortic root between the third and fourth arches, shaded in the figures. This little stretch of dorsal aortic root is the ductus caroticus, sometimes erroneously referred to as the ductus Botalli. It is the persistence of this channel on the right side, with the loss of the third as well as the first two arches, which has given us the conditions present in this particular case.

Poynter in 1916 collected seven cases of absence of the common carotid, which is a good indication of the extreme rarity of the condition. The cases are briefly as follows: Malacarne's well-known case of double aortic arch with absence of the common carotids on both sides, 1784, figured by both Quain ('44) and Henle ('76); Power's case, noted by Quain, in which the right side was involved with apparently no other anomalies; Kantor (l.c.)

states that Kosinski ('76) described the separate carotids as arising from the innominate; Gottschau ('85), separate carotids on the left side, also other arterial variations; Macalister ('86) notes in a word the divided carotids on the right side; his second case, mentioned by Poynter, we have not been able to find; v. Angermayer ('06) described a simple case on the left side.

The rarity of the divided origin of the carotids is but an expression of the constancy with which the ductus caroticus becomes obliterated. This is one of the most constant features in the varying modifications undergone by the aortic-arch system in higher vertebrates. Remnants of all the arches—except possibly the fifth—as well as of the entire ventral and dorsal aortae persist in a variety of forms, but only very rarely does the ductus caroticus escape obliteration. This little bit of vessel does not seem to have received the attention which it deserves, its early and practically invariable disappearance is quite as significant as the equally invariable presence of the fourth arch. It is represented in almost all accounts of the aortic arches since the early representations of Rathke and Boas; sometimes miscalled, it is more often left without any designation whatever.

This interruption in the dorsal aortic roots, while not invariable, is yet well established in Reptilia. The results of this break are that the blood supply of the head becomes quite independent of that of the rest of the body. Further developments effect the remaining vessels, namely, the carotids, where extensive changes may occur. Instead of the four carotids, the blood supply of the head may be taken over by one carotid alone, and this either a ventral (ext.) or a dorsal (int.) vessel. These changes have usually been correlated with the elongation of the neck, birds and certain reptiles. A very primitive, symmetrical arrangement of the aortic arches, in which both ductus carotici and both ductus Botalli persist is found in that ancient and primitive form, *Sphenodon* (O'Donoghue, '17, '20).

In reporting this case it is not our purpose to enter upon any discussion regarding the possible factors involved, general configuration of neck or trunk, or the varying position of the heart and aortic arch (O'Donoghue, Parsons, '02), in determining either the definite vascular picture or the presence or absence of anomalies.

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Resumen por el autor, A. T. Rasmussen.

Sobre la organización de la neuro-anatomía para los estudiantes de medicina, basándose en una completa base funcional, en el caso de usar para la disección un solo cerebro humano.

El autor presenta en este trabajo un programa de trabajo diario durante un cuarto del curso el cual, según varios años de experiencia demuestran, ha dado satisfactorios resultados cuando se estudia el sistema nervioso humano mediante división en funciones. Las demostraciones semanales organizadas de un modo sistemático, en las cuales se presentan preparaciones normales, experimentales y patológicas de especial interés con referencia al punto que se está estudiando, son económicas y eficientes cuando cada preparación vá acompañada de notas explicativas suficientes. El autor menciona especialmente el valor de las preparaciones con el método de Marchi, especialmente en los tractos mezclados con otras fibras. También ilustra un esquema para resumir los sistemas funcionales importantes.

Translation by José F. Nonidez
Cornell Medical College, New York

ON THE ORGANIZATION OF NEURO-ANATOMY FOR MEDICAL STUDENTS UPON A THOROUGH-GOING FUNCTIONAL BASIS WHERE ONLY THE HUMAN BRAIN IS USED FOR DISSECTION

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SIX FIGURES

The report of the proceedings of the thirtieth annual meeting of the Association of American Medical Colleges shows that there still exists great diversity in the methods used in connection with the course in neuro-anatomy. The efficient teaching of this important and difficult course is undoubtedly one of the real problems before the medical schools. This is largely because of the complex nature of the subject and the limited time allowed for its study. The most economical utilization of the student's time is also becoming extremely important throughout the entire medical curriculum, due to the expansion and increased technicality of the subject-matter. On this account any suggestions for improvement which appear to develop as a result of the application of a particular method of handling a subject should be put on record that others may test it out. Too often a teacher is conducting a course as he was taught, irrespective of the efficiency of the method.

In connection with neuro-anatomy many valuable suggestions have appeared from time to time, such as the emphasizing of the functional aspect, methods of dissection which result in maximum exposure of internal structures, construction of large fiber-tract models, providing students with outline sketches to aid them in their work on the brain stem, etc. These all have their place and should be available and utilized. What is intended here is to outline the course as it is now given at the University of Minne-

sota, in order to show the practicability of proceeding upon a thoroughgoing functional basis. It is believed that the method of approach here used has a number of features which contribute to the economical expenditure of the student's time and ultimately to greater clearness in the physiological and clinical significance of the great array of anatomical details, which are too often left as merely a bewildering complexity.

This course on the gross and microscopic anatomy of the nervous system and organs of special sense is the outgrowth of many years of teaching and research by Dean J. B. Johnston (whose functional attitude and numerous publications in neurology are well known, in particular see "A new method of brain dissection," *Anat. Rec.*, vol. 2, p. 345, 1908) together with a number of minor modifications which seemed advisable to the writer after twelve years of experience with neurology in several universities.

Since only human brains are used for dissection, the approach to various structures must be carefully planned and plainly indicated to avoid undue destruction of things to be dissected later on in the course. It is not only possible, but practicable for each pair of students to get all the essentials upon one half of a human brain and have a respectable-looking dissection at the end of the course. It is, however, quite necessary to have available in the laboratory demonstration dissections and brains cut in gross serial sections in the vertical and in the horizontal plane. Such series will last for several years if the individual sections are about a centimeter thick and each series kept together in a jar sufficiently large to permit easy removal of the brain by the students. Brains from bodies in which the blood vessels have been injected with coloring matter are often especially good for this purpose, even if they have dried up to some extent. There is frequently an excellent differentiation of the white and the gray matter due to the gray having taken up more of the pigment from the injection mass.

The general plan of the course, after an introductory survey of the meninges, external anatomy of the brain and spinal cord and the methods utilized in working out the architecture of the nervous system, is to take up in turn each of the great afferent

conducting systems, commencing in each case with the receptors or end-organs, then reviewing the peripheral nerves and ganglia involved, and finally the fiber bundles and related gray matter in the spinal cord and brain to the highest known centers. As the tracts are followed upward in the central nervous system, the principal reflex connections encountered are indicated. After considering all the afferent systems except the olfactory--which is left till later in the course when the rhinencephalon can be most advantageously dissected--the cerebral hemispheres are taken up in the lectures under such headings as cause and significance of fissure formation, cerebral localization, histology of the cortex, association, commissural and projection fibers, while in the laboratory cerebral topography and histology of the cortex are considered and as much of the hemisphere dissected as is necessary to disclose the association, commissural and projection bundles. This prepares the students for taking up the efferent mechanism, although a general idea of the efferent limb of many reflex arcs has already been obtained incidentally in connection with the afferent system. The principal efferent systems are then traced to lower motor neurones whose relation to the peripheral nervous system and muscles and glands naturally follow. The olfactory system is then taken up. This leaves for final consideration the brain ventricles and the blood supply. Since during the course of dissection the blood vessels are not readily followed, demonstration specimens with vessels injected are used in the laboratory to elucidate the arterial supply and venous drainage.

Practically all anatomical structures are considered as they are encountered in following a conducting mechanism, and hence have a meaning which cannot be conveyed by merely stating that this is concerned with one function and that with another, which is about as far as many get along the line of functional analysis. Where the course is followed by a thorough review of the functional systems, the structures take on real meaning; but why not give the student this added interest while he is working out the structure? It is also believed that by this method there is obtained a better working knowledge of the inner architecture of the brain, because it is necessary to go up and down the brain

stem several times, each time following a particular conducting chain of neurones. The method also automatically eliminates from the course unnecessary details.

Each laboratory period is preceded by a lecture which prepares the student for the practical work to follow. After the completion of a certain phase of the subject, a series of about twenty-five demonstration preparations are shown, supplementing the lecture and accompanied by notes or a fully labeled sketch or both as the case may demand. Each laboratory section is assigned a particular hour for studying these preparations. If there are at least as many specimens as there are students in a section so that each student can be kept busy, a surprisingly large amount of information is obtained by the student in an hour.

While some say that pathological material is not necessary, a very effective means of stimulating interest and at the same time a method of presenting facts that cannot be seen in normal specimens is to demonstrate the results of known pathological or experimental lesions. Series of Marchi and Weigert preparations of degenerated tracts arranged in proper sequence showing the position and extent of the most important fasciculi through the brain stem and spinal cord are almost necessary to convince some students that the specific conducting bundles that are mentioned actually exist. Experimental and pathological material stained by the Marchi method is especially valuable at the beginning of the course to show how it is possible to follow a tract a long distance even when greatly mixed with other fibers. In demonstrating a long tract, the cross-sections of various levels should be arranged in proper order and the students made to go from microscope to microscope in the direction of nerve conduction. And certainly the significance of the architecture of the normal nervous system can be emphasized in no better way before medical students than incidentally to call attention at the appropriate time to such things as syringomyelia and disassociated sensibility; degeneration of the dorsal funiculi and ataxia; equilibratory disturbances, nystagmus, etc., in vestibular and cerebellar lesions; types of blindness resulting from lesions in different parts of the optic mechanism; differences in motor

disturbances in lesions at different levels of the pyramidal system, etc.

How the subject-matter can be arranged according to the above scheme to be given in three periods (total ten hours) per week during one quarter (twelve weeks) can best be seen from the following schedule. This assumes that it is not necessary to duplicate to any extent the embryology or general histology of nervous tissue, except in the form of demonstrations here and there in the course, which is especially the case where the neurology instructor handles the neural part of the regular courses in histology and embryology. We find it desirable to have the laboratory period on which the demonstration occurs three hours in length so as to leave two hours for regular laboratory work. On this period one laboratory section at a time is taken by the instructor into the demonstration room for one hour and then returned to the regular laboratory to allow the next section to come to the demonstration.

It is, of course, not practicable to have the lectures and laboratory work run exactly parallel throughout, since the time that can profitably be spent in lectures does not vary from subject to subject directly with the time necessary to cover the practical side. Thus the first time a long conducting system is followed through the brain stem, sufficient time must be allowed to get some of the prominent land-marks and make a limited number of outline sketches illustrating typical levels. Structures not directly involved in the system followed need not be sketched in detail nor at all if not readily recognized for the time being. Like the method advocated by Doctor Lineback for embryology and used by Doctor Herrick in neurology, details are added to these drawings as subsequent systems are considered. If the particular slides to be outlined are carefully selected for the student, seven sketches will usually suffice to show all the essential internal structures of medulla oblongata, pons, midbrain, and diencephalon, including the nuclei and root fibers of the cranial nerves. For convenience these seven levels above the spinal cord are numbered from 1 to 7, from below upwards. Each student's loan collection contains many intermediate levels (from 24 to 50), which he is expected to

study. All the structures belonging primarily to a given system may be indicated, as is frequently done, by the use of a particular color.

The total number of sketches of microscopical preparations and of dissections required is twenty-eight. Copies of several charts are also called for. Naturally these must be made as simple as possible, for time does not permit the making of what some would call presentable drawings, and yet, as most agree, some sort of record seems desirable. There is, of course, no time for making microscopical preparations which students may claim as their own. Those who agree with Doctor Wells that students should come to pathology with a personal collection of normal preparations will have to show where more time is available for microscopic anatomy, since it is frequently a question of utilizing the available time in either making slides or in studying them. The statement that the loan collection is merely an easy way of getting around difficulties is unjustified in most cases, if not in all.

Two preliminary written examinations of one hour each are given during the course—one in the fourth week and another in the eighth week—sometimes in the lecture period and other times during a laboratory period, depending upon where the time can best be spared. Several short practical quizzes are given at irregular intervals. The final test consists of a two-hour written and a one-hour practical examination.

Quarterly schedule

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
1	Meninges and meningeal spaces Gross Microscopic	Meninges, gross and microscopic Dem. 1—Meninges. Cytology of nerve cells, nerve fibers, and neuroglia
2	Neurological methods General morphological Methods involving nerve degeneration	External features of spinal cord and rhombencephalon (omitting, temporarily, details on cerebellar lobes and fissures)
3	Special histological (Cajal, Golgi, etc.) Physiological Pharmacological	External anatomy of mesencephalon and diencephalon Dem. 2—Degeneration and regeneration of nerve fibers
4	Pain, touch, and temperature system commenced Tactile corpuscles	External topography of the telencephalon
5	Peripheral nerves and ganglia involved and their structure	Spinal gang., microscopic structure Begin microscopic structure of the spinal cord
6	Spinal cord Microscopic structure Reflex possibilities	Spinal cord, microscopic structure Dem. 3—Sensory nerve endings, spinal ganglia, spinal cord
7	Conduction in spinal cord of pain, touch, temperature Spinal V. tract and its relation to Nn. V., IX., X.	Lower medulla oblongata (level 1) Microscopic structure with special reference to spinal lemniscus, spinal V. tract and its nucleus
8	Spinal and trigeminal lemnisci to the thalamus Internal capsule and Cortical areas related to pain, touch, temperature	Upper medulla oblongata (level 2) and Lower pons (level 3) Microscopic structure, special reference to pain, touch, temperature conducting system
9	Deep sensibilities (muscle sense, etc.) system commenced Muscle and tendon spindles Peripheral nerves involved	Pons at entrance of N. V. (level 4) Lower midbrain (level 5) Microscopic structure with special reference to pain, touch, etc.
10	Course within spinal cord Dorsal funiculi Spinocerebellar tracts	Upper midbrain level (6) Microscopic, special reference to spinal and trigeminal lemnisci

Quarterly schedule—Continued

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
11	Cerebellum Gross structure Localization of function Microscopic structure Components of peduncles	Diencephalon (level 7), gross and microscopic series. Special reference to spinal and trigeminal lemnisci, lateral nucleus of thalamus, internal capsule
12	Brachium conjunctivum Medial lemniscus Lateral nucleus of thalamus Cortical areas related to deep sensibility system	Review brain-stem series, noting and inserting on sketches the structures of deep sensibility Dem. 4—Marchi and special slides on tracts of deep sensibility
13	Vestibular (equilibratory) system Inner ear, general structure Vestibular part in detail	Cerebellum. Review gross structure Dissection of peduncles Microscopic structure
14	Vestibular nerve and nuclei Vestibular tracts in the brain-stem, spinal cord, and to the cerebellum	Vestibular structures followed in brain-stem series and inserted in sketches of the proper levels already outlined
15	Auditory system Ear, microscopic structure of external and middle ear, cochlea	Cochlea, microscopic structure Dem. 5—Special slides on cerebellum and ear. Models of ear. Marchi series on vestibulospinal fasciculus
16	Cochlear nerve and nuclei Lateral lemniscus Cortical connections Reflex possibilities	Tracts and nuclei of auditory system followed in brain-stem series and inserted on outlines of proper levels already made
17	Visual system Eyeball Gross structure Microscopic structure	Eyeball Dissection of beef eye Microscopic structure, excluding the retina
18	Retina in detail	Retina and optic nerve, microscopic Dem. 6—Models and special slides on the eye and optic nerve
19	Optic nerve and chiasm Optic tract and connections Visual cortex Reflex connections	Study gross and microscopic material on upper midbrain and metathalamus and insert on sketch of level 6

Quarterly schedule—Continued

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
20	General visceral afferent system End organs, peripheral nerves Pathways in cord and brain Gustatory system Taste buds Peripheral nerves involved Central connections	Review brain-stem series for Nn. VII., IX. and X, and Solitary fasciculus and nucleus and insert on sketches Dissection of solitary fasciculus Taste buds Microscopic structure
21	Cerebral hemispheres Weight with age, sex, etc. Significance of fissures	Study gross divisions of cerebral hemispheres on brains being dissected and on gross series
22	Cerebral localization	Review cerebral topography
23	Histology of cerebral cortex	Histology of cerebral cortex
24	Association, commissural and projection fibers	Histology of cerebral cortex Dem. 7—Histology of cerebral cortex
25	Somatic motor system Motor cortex Pyramidal system	Dissection of association bundles, lenticular nucleus, internal capsule pyramidal fibers
26	Cortico-pontile system Extrapyramidal system Corpus striatum	In gross brain series study form and relations of internal capsule and corpus striatum
27	Lower motor neurones Cranial nerves Spinal nerves Variations and double innervation in muscles Motor end plates	Study somatic motor structures in brain-stem series and spinal cord Dem. 8—Histology of corpus striatum and subthalamie region. Marchi series on pyramidal and rubrospinal tracts. Motor end plates
28	Special visceromotor system Visceral striated muscles General visceral efferent system Sympathetic ganglia	Study brain-stem series on visceral effective nuclei and nerves Complete brain-stem sketches Histology of sympathetic ganglia
29	Craniosacral division (autonomic, parasympathetic) Relation to prevertebral and terminal ganglia	Copy charts on craniosacral outflow showing course of preganglionic and postganglionic fibers to particular viscera

Quarterly schedule—Concluded

PERIOD	LECTURE (1 HOUR)	LABORATORY AND DEMONSTRATION (2 OR 3 HOURS)
30	Thoracolumbar division (sympathetic proper) Special relation to sympathetic trunk and vertebral ganglia	Copy chart on thoracolumbar division Dem. 9—Special slides and dissections on sympathetic ganglia, nerves, rami communicantes
31	Olfactory system Olfactory membrane, nerves, bulb, tract, central connections	Histology of olfactory membrane, and olfactory bulb Dissection of rhinencephalon
32	Ventricles of the brain Topography, significance Ependyma, choroid plexus Cerebrospinal fluid	Dissection of brain completed Topography of the ventricles Choroid plexus Caudate nucleus
33	Arterial supply and venous drainage of the brain	Blood supply of the brain and cord Dem. 10—Special preparations on olfactory system, ependyma, choroid plexus and ventricles

SUMMARIZING SCHEMES

In addition to large charts and models showing the position and relations of the structures by functional systems, schemes such as are shown below have been received with sufficient enthusiasm by our students to suggest that others might find them useful. They are necessarily too dogmatic and must be interpreted liberally in places where uncertainty still exists. Such points are, of course, covered in the lectures.

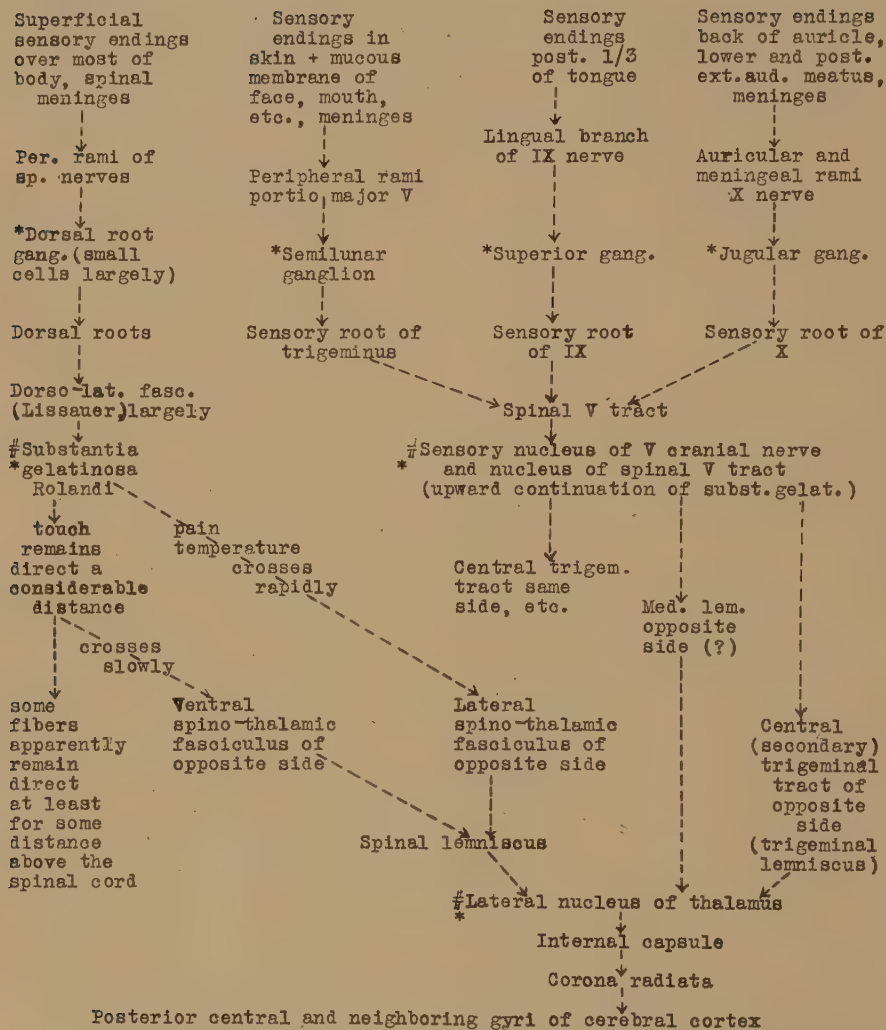
The general idea underlying the arrangement has been to make a compact and suggestive summary of each functional system. In many cases there has been sufficient space to put coordinate structures in about the same plane. The items have been so spaced and abbreviated that each system can be type-written on an ordinary-sized stencil (size $8\frac{1}{2}$ inches x 11 inches). All lines may be put in by hand or the vertical ones may be made by machine. The arrow-heads are stenciled in with a stylus shaped like a small sharp screwdriver.

It may be objected that such tabulations will be used as shortcuts and memorized at the expense of a clear idea of the position and relations within the nervous system. Students should be cautioned about this and required to pass practical examinations. On the other hand, it is a method of organizing numerous details into important units in such a fashion that it is a distinct aid to many students.

PAIN - TOUCH - TEMPERATURE

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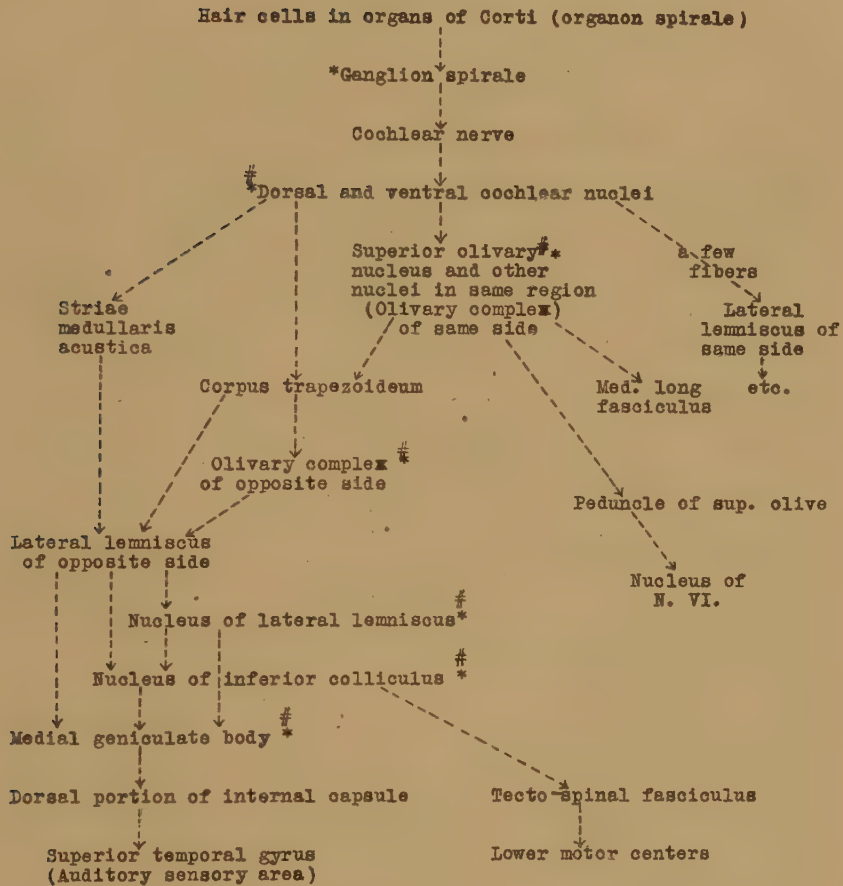
VESTIBULAR OR EQUILIBRATORY SYSTEM

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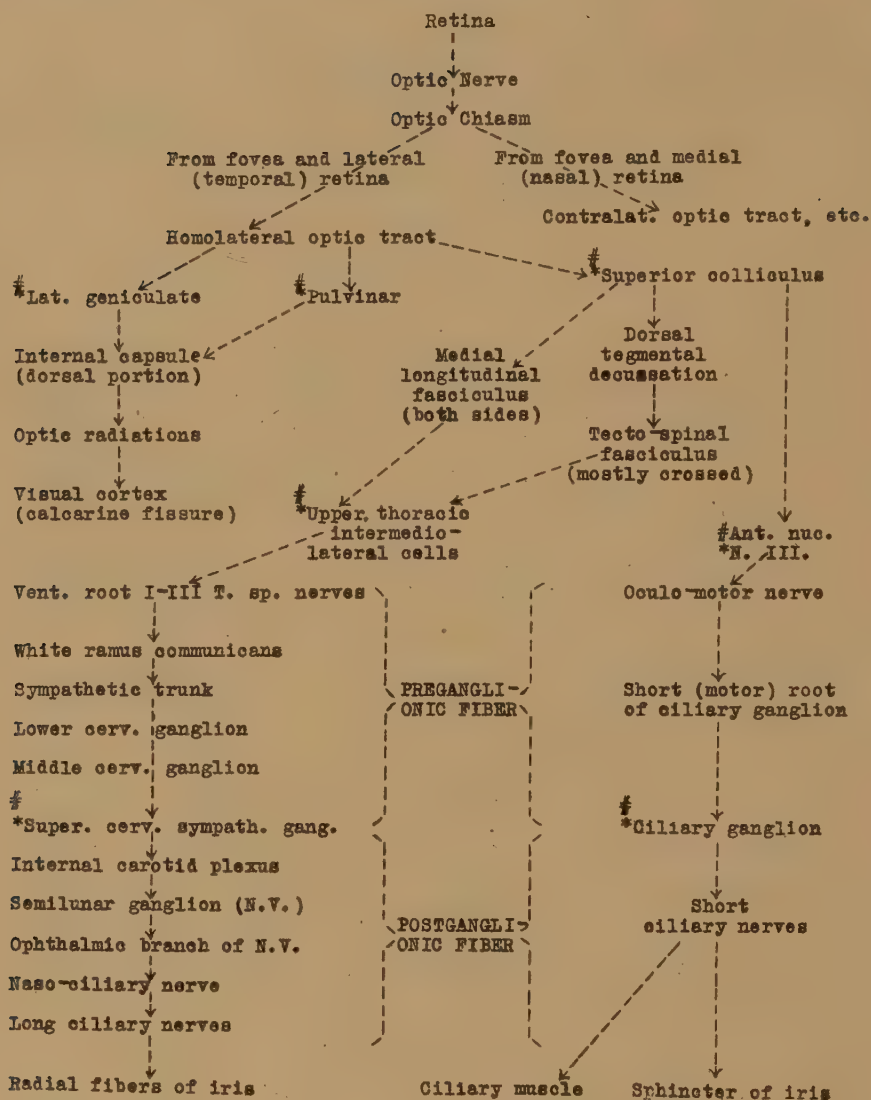
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AUDITORY SYSTEM



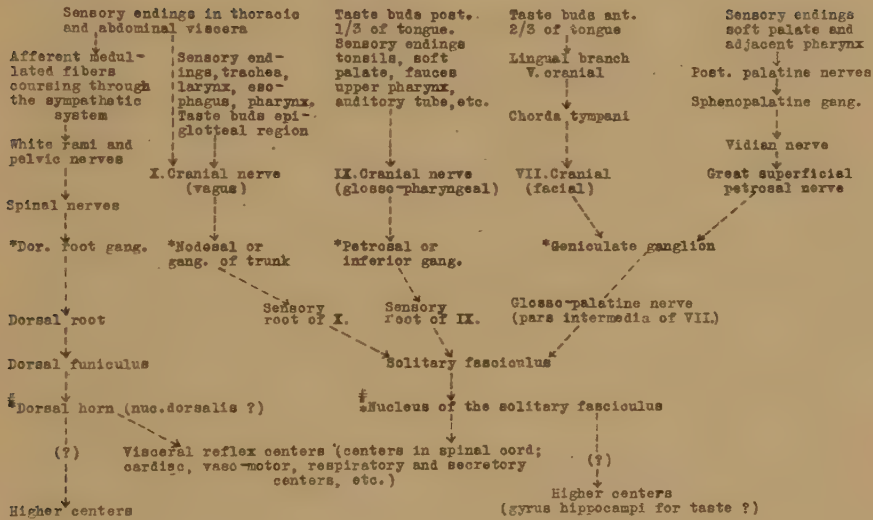
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VISUAL SYSTEM



GENERAL VISCERAL RECEPTIVE AND GUSTATORY SYSTEM.

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Resumen por el autor, Stacy R. Guild.

Un método de reconstrucción gráfica para el estudio del órgano de Corti.

La mayor parte de los investigadores han descrito las lesiones cocleares en documentos escritos acompañados de diagramas de secciones midmodiolares, estimando de modo aproximado la longitud relativa de las diversas lesiones, generalmente en términos de las partes aproximadas de las vueltas de espira, sin haber evaluado la longitud de dichas espiras. Una interpretación mejor de los resultados puede conseguirse por el método previo del autor, que consiste en representar el órgano de Corti como una banda derecha con las longitudes relativas de las medias vueltas de espira aproximadas; pero puesto que el valor linear de las diversas partes de una estructura curva contenidas en secciones radiales, oblicuas y tangenciales varía, la localización de los extremos de las lesiones solo puede ser aproximada. El método de representación gráfica que el autor describe en el presente trabajo, evalúa directamente las longitudes aproximadas de todas las porciones del órgano de Corti en los cortes seriados. En papel rayado cada espacio representa un corte; se determinan los cortes limitantes de cada media vuelta de espira y se dibuja una serie de semicírculos uniendo los puntos así hallados. El resultado es una curva semejante a una espiral que permite aproximadamente las variaciones de los valores lineares de diferentes secciones. Esta es la línea basal para representar gráficamente los datos que se desee. Los tantos por ciento de error para las diferentes porciones, determinados para seis gráficas, van incluidos en el trabajo. Los datos más exactos obtenidos por este nuevo método pueden transportarse a gráficas rectas para facilitar la comparación de numerosas observaciones. Este método no solo susministra datos más exactos sino también (en el caso de ser adoptado por otros investigadores en este campo), permite poner en posesión de otros los resultados en una forma que facilita una comparación más exacta que hasta el presente ha sido posible.

A GRAPHIC RECONSTRUCTION METHOD FOR THE STUDY OF THE ORGAN OF CORTI

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ONE CHART AND THREE FIGURES

Many papers recording the lesions found in the cochleæ of animals submitted to experimental injury by various sounds, both pure tones and detonations, have appeared in the last fifteen years. For the purposes of this communication it is unnecessary to review these, other than to mention a few of the authors. There have been several papers by Wittmaack ('07, '09, '18) and his co-workers; a series of very good works have appeared from Siebenmann's laboratory at Basel, those by Yoshii ('09), Hoessli ('12, '13), and Satoh ('17, on birds) being prominent; a long report by Hoshino ('17) on detonation injuries and more recently one by Guild ('19) on detonation injuries of exposed and protected ears. The above are only a part of the experimental sound-injury records which have appeared.

The value of these studies in the ultimate solution of the problem of the physiology of hearing is in direct ratio to the accuracy with which the position and extent of the observed lesions have been recorded; provided, of course, that the histologic technique is satisfactory. Most workers in this field have recorded their observations by individual written protocols for each animal, describing by text matter or drawings the nature of the lesions and indicating their extent and position by such very general terms as "upper part of the basal turn," "almost all of the second turn," or "lower half of third turn and upper part of second turn." Such records are, at best, very indefinite, as they make a comparison of extents of injured areas almost impossible, as will become evident later. No attempt is made in such reports to indicate the

difference in length of the various turns, and it will appear later in this paper that only the very roughest of estimates could be made thus of the relative linear values of the different lesions. They do, of course, serve to indicate in a general way the relative positions of the various lesions in the cochleæ, and have been of great value thus. The only attempts at graphic summaries of the results have been diagrams of midmodiolar sections with the tone designation or the name of the gun (Hoshino) affecting the general region indicated in the various levels of the cochlear duct shown in the section (fig. 1).

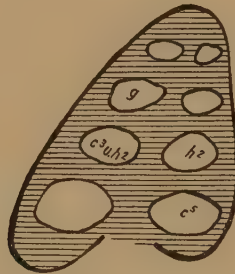


Fig. 1 Copy of a text figure in article by Yoshii ('09). This is an illustration of the method referred to in the text of diagrammatic localization of lesions with midmodiolar sections. It has been employed in connection with individual protocols and gives but a very rough idea of relative extents and positions of lesions.

While working on the problem of war deafness and the effectiveness of protective measures against detonation injuries, the writer recognized the need for a more accurate recording of the position and extent of the lesions and the desirability of having the data in a form which renders possible a more exact comparison of one record with another. While the method presented in the present communication was worked out then in its general features it was only partly made use of because of the need for haste at that time and the fact that the determination of the relative efficiencies of various forms of protection of the ears does not require the degree of accuracy in the location of the lesions that the work on the physiology of hearing requires. The relative lengths of

the various half-turns of the organ of Corti of the animals used (guinea-pigs) were approximated by actual measurements of one cochlea, and the organ of Corti represented graphically by parallel spaces in charts, with the various half-turns marked off by vertical rulings. By shades of gray and other symbols, the nature of the lesions in the various parts of the different cochleæ was shown, but the positions of the ends of the areas injured were only approximations, although serial sections were used. The reason for this is self-evident when one recalls the continuous change in linear value of each section of the organ of Corti as it

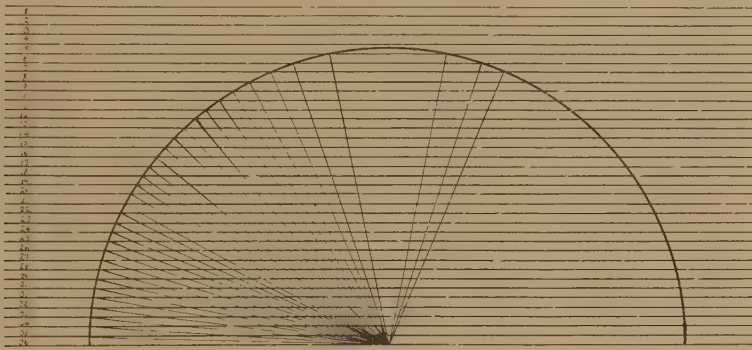


Fig. 2 Diagram to illustrate the changing linear value of the parts of a curved structure such as the organ of Corti included in sections of the same thickness. This diagram shows at a glance why the simple counting of the number of sections through which lesions pass gives but a very rough idea of the relative lengths.

passes from 'radial' to 'tangential' sections (fig. 2). A simple count of the number of sections does not give the relative lengths of any two parts of the given organ of Corti. But the method of recording used in that report (Guild, '19) permitted of a much better comparison of the lesions in various cochleæ than would previous methods not only by the author, but by any one else interested. (Chart 1 is reproduced herefrom the author's previous publication to illustrate the method.)

The method of determination of the position and extent of lesions of the organ of Corti which will now be presented is one which the author believes will improve the accuracy of the state-

Animal Side	Neck- ator Part	1 st Half-turn	2 nd Half-turn	3 rd Half-turn	4 th Half-turn	5 th Half-turn	6 th Half-turn	7 th Half-turn	8 th Half-turn (on opposite)
16 Rt.									
21 Rt.									
22 Rt.									
23 Rt.									
24 Rt.									
25 Rt.									
26 Rt.									
27 Rt.									
28 Rt.									
29 Rt.									
30 Rt.									
31 Rt.									
32 Rt.									
33 Rt.									
34 Rt.									
35 Rt.									
36 Rt.									
37 Rt.									
38 Rt.									
40 Rt.									
41 Rt.									
42 Rt.									
43 Rt.									
44 Rt.									
45 Rt.									
46 Rt.									
47 Rt.									
48 Rt.									
49 Rt.									
50 Rt.									
51 Rt.									
52 Rt.									
53 Rt.									
54 Rt.									
55 Rt.									
56 Rt.									
57 Rt.									
58 Rt.									
59 Rt.									
60 Rt.									
61 Rt.									

Chart 1 Copy of a chart in article by Guild ('19). In this method of recording the relative lengths of the several turns have been approximated, so that better estimates could be made of the lesions. Such a chart as this may also be employed in order to group and thus facilitate comparison of the more accurate data obtained for each cochlea by the graphic method described in this paper. (The various symbols and shades of gray indicate types of lesions, for the interpretation of these reference should be made to the original paper.)

ments of location and relative extents of the injuries from various causes and which will, if adopted by other workers in this field, render the results of all available to the others in a form which will permit of ready comparison. This would, without question, be desirable and would facilitate the solution of the problem of the physiology of hearing.

METHOD OF THE GRAPHIC RECONSTRUCTION

Prerequisites to the use of the method are, 1) good fixation and embedding of the cochleæ, and, 2) serial sections of these cochleæ in the approximate plane of the modiolar axis. This is the plane in which cochleæ are most often cut for study. Loss of sections or lack of uniformity in thickness of the sections interferes with the use of this method, just as such imperfections render any reconstruction method unsatisfactory. The method most used by the author in preparing cochlear material has been fully described elsewhere (Guild '19). In brief, it consists of injection fixation with warm Zenker formalin solution followed by double embedding in celloidin and paraffin: most series have been cut at $7\ \mu$; this, being about the thickness of the hair cells of the guinea-pig cochleæ, gives preparations which are better for most purposes than either thicker or thinner ones.

On closely ruled paper, such as that known as 'profile paper,' which has 400 spaces in a 20 inch width, each space is considered as representing a section, and a preliminary to starting a given reconstruction is the assigning of definite designations to as many spaces as there are sections involved in the part of the series to be plotted. The author has done this by actually writing the slide, row, and section number (in the row) in a column at the left end of the paper, rather than by designation by serial numbers of the total series of sections. This makes it very easy to identify corresponding sections and spaces at any time. With the guinea-pig cochleæ cut at $7\ \mu$, the basal turn of the organ of Corti (not the bony wall of the cochlea) usually extends over something more than 300 sections. As a 'base-line' the center of the union of the outer and inner pillar cells has been selected. The exact sections in which this union of the pillar cells is cut tangentially are deter-

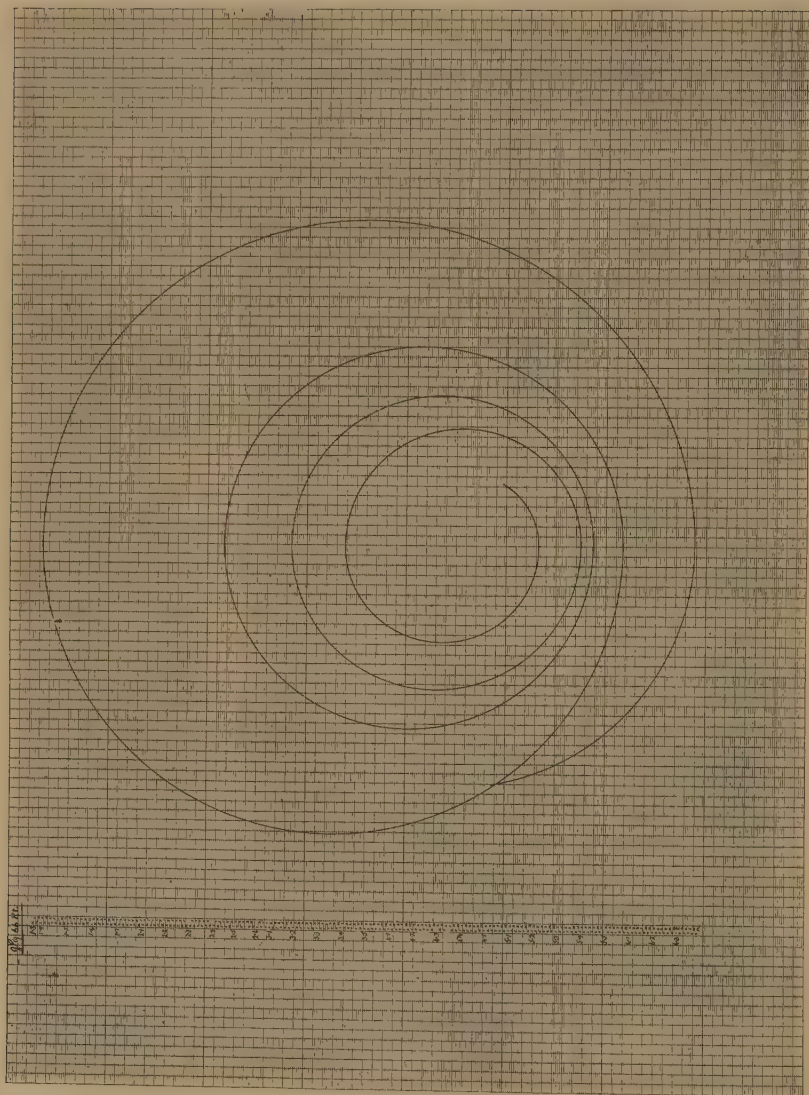


Fig. 3 Graphic reconstruction of the line of union of the outer and inner pillar cells of the organ of Corti in a given cochlea (guinea-pig no. 66 Rt.). (Reproduced at $\frac{1}{3}$ actual size of graph). This line serves as the base line for charting any desired data of the organ of Corti. Secondary concentric lines may be drawn at the correct distances to indicate rows of hair cells. Each space of the ruled paper represents a section of the cochlea, and definite designations are given the spaces. The paper used has 400 spaces in a width of 20 inches. For the method of drawing graph see text matter, page 145.

mined and indicated by marks in the correct spaces along a central line at right angles to the spaces representing sections. The tangential cuts of the organ of Corti are designated as the junction regions of the various 'half-turns' into which the cochlea may be considered as divided for descriptive purposes. Then the various marks representing the graphic positions of the half-turn junctions are connected by semicircles, each semicircle representing a half-turn of the cochlea; the centers of the semicircles are all on the common central line and the curvatures of successive circular arcs are such that there is no abrupt break evident to the eye. The graphic representation of the fractional turns beyond the last tangential cuts of the organ of Corti must be determined by a somewhat different method, since they end away from the central line. For each end the procedure is the same: the section in which the last union of pillar cells occurs is found and the distance measured by the usual microscopic methods between this union and the union of pillar cells of the full half-turn continuous with it (being first half-turn for the basal end and eighth half-turn for the apical end in the guinea-pig). This distance is multiplied by the scale of the graph and then laid off along the space of the graph which represents the given section, one end being at the place where the line of the full half-turn concerned crosses the space, and the other end on the opposite side of the central line. This determines the position of the end of the fractional turn on the graph; the other end of this fractional turn is of course where the adjacent half-turn joins it at the central line. A circle is now determined which has its center on the central line and a radius such that the circumference passes through both of the above points, and an arc drawn ending at these points. The fractional turn at the basal end has been designated the 'vestibular part' and that at the apical end the 'apical part.'

The resulting 'base-line' is a continuous curved line somewhat like a spiral in general appearance, although far from it mathematically (fig. 3). The author fully realizes the sources of error introduced: among these are: 1) the change in curvature in the cochlea is probably continuous rather than abrupt; 2) the half-turns are not true semicircles, and 3) the fact that no allowance

has been made for the 'basi-apical' distance traversed in the different parts. However, the author believes that a more exact approximation to a true graphic representation of length of the organ of Corti would involve so much mathematical calculation in the three dimensional determinations of points and their projections onto a plane surface that but few, if any, otological workers would have either time or facilities to carry out the procedure; whereas, the method here described requires only the use of microscope, drawing-board, ruled paper, and compasses, and is quickly done.

With such a base-line established, similar 'concentric' secondary lines may be drawn at the graphic distances of the various rows of hair cells, and the spaces between these concentric lines regarded as occupied by the hair cells. Then, in whatever order the serial sections are studied, the condition of the organ of Corti in each turn of each section, even to the individual hair cells, may be recorded by any suitable notation of colors or hatching.¹ By this method the position and extent of the various lesions present are determined to a degree of accuracy hitherto not attained in the recording of such lesions, and to a degree which will permit much better correlation of the work by all interested in the problem of the physiology of hearing.

CALCULATION OF ERRORS

The writer has determined the approximate error of this method of reconstruction of the organ of Corti by the following means. The magnification at which any graph is made is determined by the ratio of the thickness of the sections of the series to the width of the spaces of the ruled paper. In any section in which two places of cutting of the union of the pillar cells are less than a half-turn apart the actual distance may be measured by the usual microscopic methods and compared with the distance on the graph between the places where the line representing the union of the

¹ In order to avoid the confusion of the hatching lines by the rulings of the paper, the author plans to use tracing paper over the profile paper; this will make it much easier to disregard the section lines of the paper and yet permits them to be seen at any time for the determination of location.

pillar cells crosses the space representing the given section. This is of course a comparison of the chords subtending the arcs of the organ of Corti and of the graph rather than a comparison of the arcs themselves, but it seems reasonable to assume that the ratios of arcs and chords of these but slightly differing curves are such that the error introduced here is well within the limits of accuracy of the method in other respects. In order to determine the percentage value of the errors, the actual distance along a section was multiplied by the scale of the graph and this figure used as the denominator of a fraction of which the actual length along the corresponding space on the graph is the numerator. Evaluation of this fraction and the proper conversions give the result in the usual percentage terminology.

In order to determine the probable errors inherent in the method the writer has measured, by the procedures above described, the chords subtending the arcs of the base-line in either each tenth or each fifteenth section in each turn of each of six reconstructions. In table 1 are shown the detailed figures for one series; and in table 2 are shown only the figures giving percentage of error found by each measurement in the six series. In these tabulations, in the listing of the figures for each turn, the order of the sections measured is always the same; the order being from the tangential toward the radial or modiolar sections. This arrangement of the data calls attention to an interesting fact: i.e., the change in percentage of error is usually a continuous one and the direction of this change in value, when arranged with tangential sections first, is usually from positive numbers toward negative numbers (plus toward minus) whether the zero point is crossed or not. So constant is this that after it was observed the finding of a number out of order raised a question of error in computation and a complete recalculation was made. As a result those that are out of this order in the tables have all been rechecked until the writer is certain that they are not errors in computations. It is entirely possible that this progressive change in the percentage of error in each turn is due in some way to the semicircle combinations used in plotting.

TABLE 1

Figures on which are based the percentages of error for the parts of the graph of the right cochlea of guinea-pig no. 66 (see text matter, p. 148, for explanation)

MEASURED FROM	SLIDE, ROW, SECTION	ACTUAL DISTANCE	ACTUAL DISTANCE MULTIPLIED BY SCALE OF GRAPH	DISTANCE ON GRAPH ALONG LINE OF SECTION	PERCENT- AGE OF ERROR IN GRAPH
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
Vestibular part to 1st half- turn	6-2-11	0.713	129.4	129.5	+0.05
	6-2-1	0.969	175.9	182.5	+3.77
	6-1-3	1.177	213.6	220.7	+3.35
	5-4-12	1.429	259.3	262.8	+1.35
	5-3-9	1.598	289.9	294.5	+1.57
	5-2-6	1.729	313.6	318.4	+1.29
	5-1-3	1.831	332.3	335.8	+1.06
	4-5-5	1.902	345.1	345.1	0.00
1st half-turn to 2nd half-turn	1-4-9	0.704	127.7	125.0	-2.13
	1-5-6	1.075	195.1	184.5	-5.43
	1-6-3	1.324	240.1	225.8	-5.97
	1-7-5	1.601	290.5	272.5	-6.19
	2-1-8	1.807	327.8	307.5	-6.21
	2-2-11	1.967	356.9	334.5	-6.26
	2-4-2	2.093	379.7	355.2	-6.46
	2-5-6	2.206	400.3	371.3	-7.24
	3-1-3	2.294	416.1	382.8	-8.01
	3-2-6	2.357	427.6	390.0	-8.78
2nd half-turn to 3rd half-turn	5-4-12	0.602	109.2	117.6	+7.73
	5-4-2	0.863	156.6	166.8	+6.49
	5-3-4	1.047	189.9	200.7	+5.71
	5-2-1	1.256	227.9	238.5	+4.66
	4-5-10	1.402	254.4	265.3	+4.30
	4-4-7	1.508	273.6	284.8	+4.08
	4-3-4	1.581	286.8	298.0	+3.91
	4-2-1	1.624	294.6	305.4	+3.66
3rd half-turn to 4th half-turn	2-5-1	0.449	81.4	84.5	+3.78
	2-5-11	0.727	132.0	134.8	+2.16
	3-1-3	0.907	164.5	167.3	+1.72
	3-2-6	1.100	199.6	200.7	+0.55
	3-3-9	1.232	223.5	222.5	-0.45
	3-4-12	1.312	238.0	236.3	-0.70
	4-1-3	1.353	245.4	243.5	-0.79

TABLE 1—Continued

MEASURED FROM	SLIDE, ROW, SECTION	ACTUAL DISTANCE	ACTUAL DISTANCE MULTIPLIED BY SCALE OF GRAPH	DISTANCE ON GRAPH ALONG LINE OF SECTION	PERCENT- AGE OF ERROR IN GRAPH
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
4th half-turn to 5th half-turn	5-3-9	0.500	90.7	95.2	+4.96
	5-2-11	0.727	131.8	134.8	+2.23
	5-2-1	0.884	160.3	161.1	+0.48
	4-5-10	1.039	188.4	187.7	-0.38
	4-4-7	1.131	205.2	203.8	-0.67
	4-3-4	1.199	217.5	211.8	-2.63
5th half-turn to 6th half-turn	3-2-6	0.437	79.4	81.0	+2.08
	3-3-4	0.662	120.1	120.8	+0.64
	3-4-2	0.809	146.7	146.0	-0.49
	3-5-5	0.954	173.1	170.2	-1.68
	4-1-8	1.039	188.5	183.6	-2.61
	4-2-11	1.086	197.0	188.5	-4.31
6th half-turn to 7th half-turn	5-3-4	0.438	79.5	78.9	-0.79
	5-2-6	0.648	117.5	114.4	-2.67
	5-1-8	0.779	141.4	136.5	-3.47
	4-5-5	0.909	165.0	156.3	-5.28
	4-4-2	0.972	176.4	165.5	-6.16
7th half-turn to 8th half-turn	3-4-7	0.337	61.2	59.3	-3.18
	3-5-5	0.545	98.9	95.5	-3.43
	4-1-3	0.669	121.3	116.1	-4.31
	4-2-1	0.743	134.8	128.2	-4.92
	4-2-11	0.785	142.5	134.8	-5.40
8th half-turn to apical part	5-1-8	0.302	54.9	58.6	+6.83
	5-1-3	0.400	72.5	77.0	+6.20
	4-5-9	0.484	87.8	87.8	0.00

In studying table 2 it is observed that the general nature of the differences between the measurements of the actual cochlear sections and of the graph for the corresponding turns of the several cochleæ is the same. Thus, for the measurements between parts of the first and second half-turns the figures giving the percentage of error are in each case negative, and are larger in the modiolar region than any other figures for the same cochlea. This simply means that the true curvature of the basal turn differs

TABLE 2

Percentages of error as determined for each of six cochleae (see text matter, p. 149, and table 1 for explanation)

	ANIMAL NUMBER AND SIDE					
	64 Left	65 Right	66 Right	67 Right	68 Right	69 Right
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Vestibular part to 1st half-turn	+4.47	-1.20	+0.05	+ 4.25	- 1.36	+13.28
	+1.62	-1.22	+3.77	+ 5.13	- 0.05	+ 9.47
	+0.67	-2.18	+3.35	+ 4.16	+ 0.97	+ 7.56
	+0.55	-0.95	+1.35	+ 3.61	+ 1.49	+ 6.17
	+0.81	-0.88	+1.57	+ 2.73	+ 1.75	+ 3.38
	+0.50	-0.00	+1.29	+ 1.49	+ 1.47	+ 2.37
	0.00	-0.07	+1.06	0.00	+ 1.24	0.00
		0.00	0.00		0.00	
1st half-turn to 2nd half- turn	-4.62	-2.40	-2.13	- 8.01	-10.76	-10.28
	-5.13	-3.77	-5.43	- 9.38	-10.21	-11.14
	-6.29	-5.08	-5.97	- 9.17	- 9.78	-11.62
	-6.47	-5.04	-6.19	- 9.74	- 9.71	-11.55
	-6.99	-5.36	-6.21	-10.32	- 9.03	-11.65
	-7.05	-5.52	-6.26	-10.71	- 9.18	-12.28
	-7.76	-5.58	-6.46	-11.49	- 9.70	-13.20
	-8.39	-6.80	-7.24	-11.87	-10.41	-14.08
	-8.62	-7.44	-8.01	-13.12	-10.87	
2nd half-turn to 3rd half- turn	+6.23	+6.92	+7.73	+ 7.85	+ 1.90	+ 4.87
	+5.54	+4.08	+6.49	+ 5.75	+ 2.05	+ 4.57
	+4.38	+4.56	+5.71	+ 3.98	+ 2.02	+ 2.64
	+4.01	+4.28	+4.66	+ 3.73	+ 1.92	+ 2.39
	+3.65	+3.18	+4.30	+ 3.29	+ 1.58	+ 2.27
	+3.32	+2.65	+4.08	+ 2.75	+ 1.49	+ 2.21
	+3.00	+2.94	+3.91	+ 2.47	+ 1.36	+ 1.33
	+2.33	+2.81	+3.66			+ 1.17
3rd half-turn to 4th half- turn	+1.86	+4.39	+3.78	- 0.62	+ 4.42	- 3.08
	+0.17	+3.02	+2.16	- 0.88	+ 2.06	- 1.81
	-0.57	+1.51	+1.72	- 0.58	+ 0.11	- 1.36
	-0.88	+1.34	+0.55	- 1.26	- 0.34	- 2.08
	-1.31	-0.30	-0.45	- 1.86	- 0.73	- 2.41
	-1.81	-0.92	-0.70	- 2.43	- 0.79	- 3.62
	-2.93	-1.92	-0.79	- 3.74	- 1.49	

TABLE 2—Continued

	ANIMAL NUMBER AND SIDE					
	64 Left	65 Right	66 Right	67 Right	68 Right	69 Right
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4th half-turn to 5th half-turn	-0.50	+4.17	+4.96	- 1.97	+ 2.66	- 0.38
	-1.25	+1.46	+2.23	- 3.69	+ 0.86	- 0.34
	-2.22	+0.23	+0.48	- 3.22	+ 0.70	- 0.28
	-2.97	-0.60	-0.38	- 3.70	+ 0.50	- 0.68
	-3.28	-0.82	-0.67	- 3.92	- 0.75	- 0.97
	-5.00	-1.64	-2.63	- 3.84	- 1.01	- 2.39
				- 5.36		
5th half-turn to 6th half-turn	+0.91	+2.88	+2.08	+ 2.27	+ 3.00	- 1.87
	-0.47	+0.21	+0.64	- 1.76	+ 0.76	- 2.56
	-1.64	-1.22	-0.49	- 2.31	- 0.44	- 3.21
	-1.88	-2.30	-1.68	- 3.52	- 1.58	- 3.42
	-2.65	-3.34	-2.61	- 4.40	- 2.85	- 3.94
	-3.63	-5.71	-4.31	- 5.51	- 3.78	- 4.98
				- 6.37		
6th half-turn to 7th half-turn	-0.26	+2.37	-0.79	- 4.87	+ 3.45	+ 3.89
	-2.58	+0.49	-2.67	- 5.61	- 0.56	- 0.10
	-3.73	-1.05	-3.47	- 6.57	- 0.86	- 0.22
	-3.81	-3.06	-5.28	- 7.07	- 1.61	- 2.29
	-5.68	-3.39	-6.16	- 7.35	- 2.68	- 3.73
7th half-turn to 8th half-turn	-2.99	+1.66	-3.18	- 5.43	+ 2.03	+ 4.60
	-4.69	-2.26	-3.43	- 6.58	- 0.83	+ 0.17
	-5.67	-4.00	-4.31	- 8.38	- 4.75	- 0.72
	-5.87	-4.52	-4.92	- 9.69	- 5.72	- 2.43
	-6.99		-5.40	-10.89	- 7.92	
8th half-turn to apical part		-0.33	+6.83	+ 7.04		+ 3.35
		-1.34	+6.20	+ 2.99		- 2.08
		-0.38	0.00	0.00		+ 1.39
		0.00				0.00

more from a circular nature than any other part does, and that the degree of curvature is less than that of a circular arc of the dimensions of that determined by this graphic method. On the other hand, the figures for the second to third half-turns are in each case positive, and the percentage of error much less than for the first to second half-turns; this indicates that in this region the curvature

of the organ of Corti deviated from the graph in the direction of having a slightly greater degree of curvature than the circular arcs of the graph. In the remaining turns there is more variation in the individual cases, but the most common result is that of having the tangential sections with a positive error which grows less and crosses the zero point into negative values toward the modiolar sections.

In the actual use of the method in determining the extent and position of lesions in given cases, a correction factor could be determined and applied for the portions of the given cochlea, which ought to give values accurate enough to enable recognition of any numerical ratios favoring any of the theoretical requirements of the several theories of hearing.

COCHLEAR VARIATIONS

An interesting secondary result of the method is the demonstration of the amount of variation of individual cochleæ, both in total size and in relative proportions of the various parts. This is best brought out by tabulating the number of sections through which the various half-turns extend. Such a tabulation is given in table 3, the figures of the table being the number of sections from one tangential cut of the union of pillar cells of the organ of Corti to the next tangential cut of the same part; except for the vestibular part and the apical part, where the last ones are not cut tangentially. Variations in the orientation of the blocks for sectioning may be the cause for some of this, but the writer thinks that the orientation was too similar in the several series to account for very much of it; and, further, if this were the cause, the variation would be in the same direction for all turns, which it is not. Even the few cases recorded are sufficient to show that it is not at all correlated with the weight of the animals, the figures for which have been included in the tabulations. For instance, the smallest animal, weighing 225 grams, has a cochlea in which the distances between the several adjacent tangential cuts of the union of pillar cells are greater than for the corresponding turns of the largest animal, weighing 350 grams, and the variations are not uniform.

COMPARISON OF VARIOUS RECORDS

To facilitate comparison of the lesions in a large number of cochleæ, the positions and lengths determined by this method may be transferred to charts such as the author used in recording the lesions of the detonation deafness experiments. One of these is reproduced here as chart 1 for purposes of illustration. In such a chart the relative lengths given each half-turn are those of an

TABLE 3

Variation in individual cochleæ. The figures show the number of 7μ sections included in each turn (see text matter, p. 154, for parts measured)

	WEIGHT OF ANIMAL IN GRAMS						
	320	320	285	340	350	255	225
Animal number and side.....{	64 Left	64 Right	65 Right	66 Right	67 Right	68 Right	69 Right
'Vestibular' part.....	80	80	73	101	74	105	99
1st half-turn.....	314	307	321	329	286	316	287
2nd half-turn.....	265	269	266	293	271	278	281
3rd half-turn.....	199	197	192	201	187	200	198
4th half-turn.....	172	175	176	186	169	185	180
5th half-turn.....	156	148	146	152	140	153	148
6th half-turn.....	145	143	139	146	134	147	140
7th half-turn.....	116	114	113	119	108	118	112
8th half-turn.....	83	84	86	98	87	94	88
Apical part.....	20	21	31	46	14	30	52

average cochlea; so there would be, of course, some slight adjustments necessary in fitting each cochlea to the fixed length, but the gain in facility of comparison might in some cases render it advisable. The original graphic plots can of course be reproduced as either line drawings or halftones.

SUMMARY

Most observers have reported cochlear injuries by written protocols and midmodiolar section diagrams, making only rough estimates of the relative lengths of various lesions, usually in terms of approximate parts of turns, without evaluation of the lengths of various turns. Better interpretation of results is permitted by

the author's former method of charting the organ of Corti as a straight band with the relative lengths of the various half-turns approximated, but since the linear value of the several parts of a curved structure contained in radial, oblique, and tangential sections varies, the locations of the ends of lesions were only approximate. The graphic reconstruction method here presented evaluates directly the approximate lengths of all portions of the organ of Corti when cut in serial sections. On ruled paper each space represents a section; the limiting sections for each half-turn are determined and a series of semicircular arcs drawn connecting the points thus found; the result is a 'spiral-like' curve making approximate allowance for variations in linear values of different sections. This is the 'base-line' for charting any desired data. The percentages of error for different portions have been determined for six graphs and are recorded.

The more accurate data secured by this new method may be transferred to straight-line charts to facilitate comparison of numerous observations. The method will not only yield more accurate data, but will, if adopted by other workers in this field of investigation, also render the results of all available to the others in a form which will permit of more ready comparison than has previously been possible. This would, without question, greatly facilitate the solution of the problem of the physiology of hearing.

The general method may be modified so that other parts of cochlea may also be graphically represented. The demonstration of variation in individual cochleæ is a secondary result.

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Resumen por el autor, Oscar V. Batson.

La coloración diferencial del hueso.

I. El teñido de ejemplares conservados.

El autor ha probado una serie de derivados de la alizarina y mordientes con el propósito de hallar, si esto fuese posible, un colorante que ofrezca una coloración de contraste con la obtenida por el rojo de rubia ordinario. Para los experimentos ha empleado embriones de pollo y cerdo. El material fué fijado en formol al 10 por ciento. Para eliminar el color de la sangre se empleó una solución débil de peróxido de hidrógeno. La solución tintórea fué una solución saturada del colorante en alcohol de 95°, una parte, y veinte partes de alcohol de 95°. Los ejemplares fueron diferenciados en una solución al medio por ciento de ácido sulfúrico en alcohol de 95°, después de lo cual fueron deshidratados y aclarados según el método de Spalteholz. Los veinte colorantes que pudieron obtenerse fueron los siguientes:

Negro de alizarina ácido, R. Meister, Lucius & Brüning	Pardo de alizarina RB, polvo, Fr. Bayer & Co.
Negro de alizarina SR, polvo, Fr. Bayer & Co.	Negro de alizarol 3G, Nat. Aniline and Chemical Co.
Azul de alizarina Br3g, polvo, Fr. Bayer & Co.	Carmín de índigo, Grüber
Negro de alizarina Bayer NR, Polvo, Fr. Bayer & Co.	Ácido carmínico, Grüber
Negro de alizarina SBB, polvo, Fr. Bayer & Co.	Rojo de Burdeos, Grüber
Pardo de alizarina AR, polvo, Fr. Bayer & Co.	Naranja, Grüber

THE DIFFERENTIAL STAINING OF BONE

I. THE STAINING OF PRESERVED SPECIMENS¹

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The study of ossification centers, of healing in fractures, and of bone growth in general has been greatly facilitated by the use of alizarin in its natural and synthetic forms. That madder root when fed to animals colors the bone red has long been known. It seems that Belchier (1736) rediscovered this fact and brought it to popular attention. Almost 150 years earlier, however, Laevinus Lemnius (1581) mentioned this property of madder root.² Considering the numerous editions and translations of Lemnius' "Hidden Miracles of Nature," it seems remarkable that no use was made of this observation.

Biologists and chemists have examined madder root to find the active staining principles. The chief of these have been found to be alizarin and purpurin, mainly the former. Since the pure synthetic alizarin has been available it has been largely used in the staining of preserved specimens, for the demonstration of the skeleton. This staining technic coupled with clearing methods has been presented in detail by Lundvall ('04, '05) and Spalteholz ('14). Alizarin in the form of its soluble salt, sodium alizarin monosulphonate, has also been used to supplant madder root in the vital staining of bone. Gottlieb ('14), in a very thorough paper, describes the use of the above salt for vital staining. Brooks ('17, '20) more recently has reported a series

¹ A series of specimens stained with the described dyes was demonstrated at the meetings of the American Association of Anatomists at The Wistar Institute, Philadelphia, March, 1921. This demonstration included a set that had been prepared in August, 1920.

² " . . . erythrodanum seu rubea, qua ossa pecudum sandicino rubentique colore imbuunt, . . . " De Miraculis Occultis Naturae, p. 390, lines 30 and 31.

of studies in which the same salt was used, and it is quite probable that sodium alizarin monosulphonate is now used in most laboratories for vital staining of bone.

Graebe and Lieberman first produced alizarin synthetically in 1868. Since that time many synthetic alizarin derivatives have been produced by the dye chemists. A glance at the catalogs of the dye firms reveals alizarin reds, browns, blacks, blues, greens, and yellows. Apparently none of these have been used for the successful staining of bone. Ehrlich ('85) used alizarin blue S in studying oxidation in tissues, but with this stain did not report any staining of bone. Rawitz ('96) who used alizarin extensively in microscopic technic did not mention bone staining. Spalteholz ('14) uses small quantities of alizarin cyanatum in staining preserved specimens to improve the color of the alizarin red. Gottlieb ('14) unsuccessfully tried to stain bone *intra vitam* with alizarin blue and alizarin green (brand or formula not mentioned).³ In reporting Bardeen's technic for the study of ossification centers, Hill ('06) describes the former's use of alum carmine, a dye of a wholly different chemical series. Miller ('21) in a recent paper refers to the same work.

In certain types of bone work a stain affording a contrast to the red-brown of the usual alizarin would seem desirable. It might be possible to alternate the red with a contrasting color in *intra vitam* work, or it might be possible to bring out more detail after preservation by the use of a counterstain for that part of the bone not colored during life. With the view of possibly finding such a stain, the following study of alizarin derivatives and mordant dyes was begun.

Among the readily soluble alizarin cloth dyes available those were selected which gave promise of affording a contrast to the alizarin red. The non-alizarin mordant dyes of the histological laboratory were tried out, partially to see if the anthraquinone grouping (present in the alizarins) is an essential element of a bone stain and partially in the hope of finding a more brilliant bone stain.

³ "Ich möchte hier noch kurz erwähnen, dass ich mit einer Reihe verwandter Farbstoffe (Alizarinblau und Alizarin grün) nach dieser Richtung Versuche angestellt habe, sämtlich aber negativ ausgefallen sind" (S. 192).

In investigating the properties of the various stains the following technic was used. The tissue consisted of portions of pig embryos ranging in size from 3.5 cm. to 8 cm. These embryos were part of the laboratory stock obtained from the packing house fixed in 10 per cent liquor formaldehyde. The blood color was removed from part of the material by bleaching in a $\frac{1}{2}$ per cent by volume solution of hydrogen peroxide (the ordinary peroxide of commerce is 3 per cent by volume), followed by removing the gas bubbles with the suction pump.

Stock solution of stain

Strain being investigated.....	250 mg.
Water.....	5 cc.
Shake well and add of 95 per cent alcohol.....	95 cc.

In no case did all of the stain go into solution.

1. Material washed in water.
2. Stained twenty-four to forty-eight hours in

Stock solution.....	1 part
95 per cent alcohol.....	20 parts

3. Differentiated in $\frac{1}{2}$ per cent solution of sulphuric acid in 95 per cent alcohol.

4. Dehydrated and cleared after the method of Spalteholz.

To the present time twelve stains have been found to combine differentially with the bony skeletal elements. A list of the stains, with a brief comment on their behavior and the results obtained, is given below.

Alizarin group

1. Acid alizarin black R. Farbwerke vorm. Meister, Lucius, & Brüning. Hoechst o/M. Stains the skeletal elements a deep reddish purple. The soft tissues and cartilages decolorize rather slowly; however, differentiation is good from the beginning.

2. Alizarin-black SR powder. Farben fabriken vorm. Fr. Bayer & Co., Elberfeld. This stain likewise imparts a purple color to the bones. Satisfactory stains may be obtained from 1 to 250 dilutions. When this is done the bone is not stained so intensely, but decolorization is easier. Must be differentiated in sulphuric acid alcohol.

3. Alizarin-blue Br3G powder. Bayer. Stain is fairly differential from the start. Bone is an azure blue.

4. Alizarin-black-Bayer NR powder. Bayer. This stain gives a good purple to the bones. The other tissues decolorize readily and the end-result is a very sharp stain. See no. 8.

5. Alizarin black SBB powder. Bayer. Bones a sharp indigo. The other tissues take this stain slightly. The differentiation is rapid and sharp. This stain seems to be one of the most satisfactory. Its color is devoid of reddish tinge.

6. Alizarin brown A R powder. Bayer. Imparts a reddish brown to bone.

7. Alizarin brown R B powder. Bayer. Very similar to no. 6.

8. Alizarol black 3 G. National Aniline and Chemical Co., New York. A new American stain, supposedly an alizarin. The shade is slightly different from no. 4, but otherwise the dye is quite similar. This is a very satisfactory stain. It is available in quantities.

9. Indigocarmine, Grübler. This stain can be used in a saturated solution or in any dilution. It decolorizes perfectly in water as well as in the acid alcohol mentioned. The resulting stain is a medium indigo. One of the easiest stains of the series to handle and one producing an excellent preparation.

10. Carminic acid, Grübler. This stain like the previous one can be differentiated in water. If the reaction of the mounting fluid is acid the color of the bone in the specimen is an orange-red, if the reaction is alkaline the color approaches a Bordeaux. If acid decolorization is used the specimen should be alkalinized before mounting. Dr. Barden's use of alum carmine was referred to earlier. It is perfectly obvious that the use of carminic acid is but a modification of this technic.

11. Bordeaux red, Grübler. This stain can be used in a saturated solution if desired, but this makes decolorization a little more tedious. The skeletal elements come out a brilliant shade of Bordeaux and if decolorized properly the rest of the structures are colorless. The end-results seems much better than those obtained with the usual alizarin red.

12. Aurantia, Grübler. Solutions near saturation work best. The bones stain a bright orange-yellow. The crystalline stain gives a deeper stain than the amorphous.

In working with the individual stains certain modifications as noted above were found to be desirable, but on the whole the standard technic was entirely satisfactory for the preliminary work. Portions of the above specimens were subjected to treatment with 5 per cent sulphuric acid in 95 per cent alcohol to test the permanence of the stains. Some decolorization (not decalcification) was noted when the time in the 5 per cent acid was extended over forty-eight hours. This permanence to sulphuric acid applies to the skeleton of the chick at hatching and to the

skeletons of pig embryos of the stages mentioned. Sulphuric acid has not been satisfactory when used on the bone of young rats. Lundval ('05) must have had in mind the acids that form soluble calcium salts when he comments, "Da das Alizarin in sauren Lösungen beinahe vollständig entfärbt wird, kann man keine von den schon beschreibenden Knorpelfarbenmethoden verwenden." Calcium sulphate is quite insoluble. It should be noted here that in differentiating specimens stained by sodium alizarin monosulphonate, the acidulation of the alcohol with sulphuric acid causes the red soft tissues to become immediately yellow, while the bone remains red. This yellow color is readily extractable. Spalteholz ('14) made use of these facts in his earlier technic by adding small amounts of acetic acid to the staining solution itself.

SUMMARY

Of these new bone stains indigo-carmin, Bordeaux red, alizarol black 3G (National Aniline and Chemical Co.) and alizarine black SBB powder (Bayer), seem particularly suitable for staining preserved specimens.

The number of stains shown to combine differentially with bone indicates that there is a large number of mordant or adjective dyes with this property.

The anthraquinone grouping is not necessary for bone staining.

Weak solutions of sulphuric acid are adequate and satisfactory decolorizing agents for soft tissues in differentiating bone stains.

Staining of bone in preserved specimens is largely a matter of differential decolorization.

I wish to thank Dr. A. G. Pohlman, of St. Louis University, in whose laboratory the preliminary experiments for this paper were carried out, and to acknowledge especially my indebtedness to Prof. R. Fischer, of the Department of Chemistry of this University, who placed a collection of alizarines at my disposal.

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Resumen por el autor, Oscar V. Batson.

La restauración del material anatómico momificado.

El material anatómico que se ha secado demasiado para la disección puede restaurarse completamente al estado fresco mediante inyección subcutánea de un líquido conservador. El líquido empleado, cuya composición no es de gran importancia en tanto que contenga una gran cantidad de agua, debe inyectarse primero en la tela subcutánea y después puede inyectarse debajo de las fascias musculares. La piel se ablanda rápidamente, y con ayuda del masaje al cabo de unos minutos aparece casi como en el vivo. Los antisépticos que persisten en el cadáver desde el previo embalsamamiento no son extraídos de los órganos, como sucede cuando se emplea el método ordinario de remojar el material seco.

Translation by José F. Nonidez
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RESTORING MUMMIFIED ANATOMICAL MATERIAL

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ONE FIGURE

Gross anatomical material is difficult to keep in good condition for dissection, even with frequent attention. This is particularly true of the hands and feet and of any region marked by bony prominences. This drying is prone to occur during storage and during holiday recesses despite precautions.

The methods commonly used to combat this evil are either to immerse the member in a weak antiseptic fluid or to cover it with cloths thoroughly saturated with such a fluid. These procedures have two disadvantages: The skin is fairly impervious to the fluid, particularly if it has dried to the point where it is board-like and translucent; either of the above methods tend to remove the preservative used in embalming in direct proportion to their efficacy in softening.

These disadvantages may be entirely overcome and the most hardened, translucent, mummified hand or foot completely restored by combining massage with the subcutaneous injection of fluid. A 25-cc. or 50-cc. metal barrel syringe, with a large needle, seems to be the most satisfactory instrument. The pressure bottle does not give as good results. The needle is passed into some fairly areolar region, and some 10 cc. of fluid injected. This softens the tissues for a small area, and by beginning at the border of this for the next injection, and by massaging the fluid around in the subcutaneous tissues the work is gradually extended. The skin softens readily when the fluid is applied from below and in a few minutes looks even life-like. The contained antiseptics remaining from the original embalming are not washed out. If the fluid injected contains a large percentage of water, the exact

nature seems immaterial. The following fluid works well and seems to aid in preventing a recurrence of drying:

Gum tragacanth.....	5 grams
Glycerine.....	100 cc.
Water up to.....	1 liter
Let stand 48 hours and filter through gauze.	

The muscles, fascias, and skin can be completely restored. Material which has been preserved with a large percentage of liquor formaldehyde does not revive so completely.



Fig. 1 The ulnar side of the forearm and hand together with the ring- and little fingers has received subcutaneous injections of the fluid recommended. The remainder of the hand is representative of the condition of the whole hand before injection.

Resumen por el autor, H. D. Senior.

La arteria comitans nervi peronaei communis.

El autor no ha podido comprobar la observación de Salvi, hecha al describir por primera vez esta arteria en 1899, con referencia a la constancia de la existencia de esta arteria en el miembro adulto. En su opinión, la arteria comitans nervi peronaei communis falta más a menudo que existe. La arteria nervi peronaei communis embrionaria descrita por De Vriese en 1902 y considerada por dicho autor como precursora de la del adulto ha sido hallada por el autor una sola vez al examinar un número relativamente elevado de miembros embrionarios con el fin de encontrarla. Cuando existe, esta arteria puede probablemente llevar a cabo, durante algún estado del desarrollo, la función asumida definitivamente en casos normales por la parte proximal de la arteria tibial anterior; esto parece haber sucedido, en efecto, en un miembro mencionado por Velpeau en 1835. La opinión de De Vriese, sin embargo, sobre la constancia de la aparición de esta arteria embrionaria y su participación en el desarrollo normal de la arteria tibial anterior parece haberse basado en el exámen de material embrionario imperfectamente conservado. La opinión ulterior expresado por dicho autor, y mantenida por Manino en 1906, con referencia a la identidad de la arterias inferolateral de la rodilla y la recurrente tibial anterior del adulto y la arteria nervi peronaei communis del embrión no parece posible bajo el punto de vista anatómico.

Translation by José F. Nonidez
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THE ARTERIA COMITANS NERVI PERONAEI COMMUNIS

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LITERATURE

In 1899 G. Salvi described and figured a branch of the popliteal artery that accompanies the common peroneal and the proximal part of the deep peroneal nerve and ends by joining the a. tibialis anterior. Salvi's description of the a. comitans nervi peronaei seems the first to have been given, although the proximal part of an abnormal anterior tibial artery referred to very briefly by Velpeau in 1835 may quite conceivably have been a vessel of the same nature.¹ Further evidence of the occasional existence of a common peroneal artery is to be found also among the numerous illustrations of dissections of the lower extremity that were published during the last century.²

In the particular case described by Salvi the a. comitans nervi peronaei communis arose from a branch of the popliteal artery from which also an a. saphena parva (or a. suralis magna) took origin. Becoming free about the level of the superior articular surface of the tibia, the a. comitans followed the course of the common peroneal nerve to the neck of the fibula and then accompanied the deep peroneal as far as the junction of the

¹ "Je l'ai vue (l'artère tibialis anterior) deux fois devenir superficielle des le milieu de la jambe. Dans l'un des ces cas, elle sortait comme à l'ordinaire de la poplitée. Dans l'autre, au lieu de traverser le ligament interosseux, elle se contournait en dehors du péroné et suivait le trajet du nerf musculo-cutané." Velpeau ('35), p. 44.

It is unfortunate that no specific mention is made of the relation borne by the proximal part of the artery of the second case to the common peroneal nerve.

² See, for example, Quain ('44), pl. 78, fig. 1; Hyrtl ('66), taf. 3, fig. 3; Ellis and Ford ('76), pl. 53.

proximal and middle thirds of the tibia, where it joined the anterior tibial artery. The *a. comitans nervi peronei* gave origin to two long slender branches: the first, arising opposite the neck of the fibula, followed the lateral aspect of that bone to the lateral malleolus, where it anastomosed with the other arteries in the neighborhood. The second, arising near the terminal end of the parent vessel, became the *a. comitans nervi peronei superficialis*, which was also described for the first time in this publication. The case described in Salvi's paper is illustrated by his figures 3 and 4; figure 1 illustrates a vessel of an almost precisely similar nature that occurred in another limb in which the *a. saphena magna* was present also. The author states that the accompanying arteries of the peroneal nerves are present in a more or less well-developed state in all individuals (p. 37).

Since the publication of Salvi's paper, Manno has described *arteriae comitantes* of the peroneal nerves as occurring in five other human lower extremities. In 1906 the latter author described an adult limb in which the ischiadic artery had persisted and in which the common peroneal artery was present also. In the same year he published an extended study of the relations of the common peroneal artery of vertebrates in general, which contained descriptions of the peroneal *arteriae comitantes* of four other human limbs. The so-called common peroneal artery illustrated in figure 2 of the latter paper appears to be nothing more than an unusually large anastomotic connection between the *a. genu inferior lateralis* and the *a. recurrens tibialis anterior*, and is described as such.³ The peroneal *arteriae comitantes* of the other cases seem to resemble closely those described by Salvi.

In connection with Manno's identification of the vessel illustrated in the figure just referred to, it may be remarked that, inasmuch as the inferior lateral genicular artery of the adult

³ "Nell' arto addominale destro di un bambino di un mese, che ho riprodotto nella figura 2 si trova un'arteria peronea communis molto evidente formata dall' anastomosi di un ramo discendente dell' arteria genu lateralis inferior e di un ramo ascendente dell' arteria recurrens tibialis antica. Essa decorre parallela al nervus peroneus communis, ma un pò al di sopra di questo." Manno ('06 a), p. 273.

limb is separated from the common peroneal nerve by the lateral head of the gastrocnemius muscle and by the plantaris, it cannot be accepted as the equivalent of the proximal part of the a. comitans nervi peronei communis described by Salvi. The fact that the channel of connection between the popliteal and anterior tibial arteries formed by the anastomosis between the a. genu lateralis inferior and the a. recurrens tibialis anterior pursues a course entirely different from that followed by the a. comitans nervi peronei communis, is brought out clearly by Salvi's figures 1 and 4, in which the two channels of connection between the popliteal and the anterior artery are shown to coexist. Manno is not only willing to accept the a. genu inferior lateralis as representing the proximal part of the a. comitans nervi peronei communis, but seems willing also to accept the a. propria articularis capituli fibulae of Weber in a somewhat similar light.⁴ The latter artery has very little in common with the a. comitans nervi peronei communis, for Weber's description indicates quite clearly that it is separated from the common peroneal nerve not only by the gastrocnemius and plantaris muscles, but also by the soleus.⁵

DeVriese's conception of the embryonic history of the a. comitans nervi peronei communis is set forth in the description of the development of the arteries of the human extremities, published by that author in 1902. The occurrence of the a. comitans nervi peronei communis in the adult limb is described as resulting from the persistence of an embryonic artery named the a. nervi peronei communis (p. 719). The a. nervi peronei communis is described as a branch of the a. ischiadica that follows the course of the common peroneal nerve and per-

⁴ "Quindi l'arteria capituli fibulae di Weber, sia per l'origine sua dalla poplitea, che per il tragitto parallelo al nervo peroneo communis, è da considerare piuttosto come una ridotto arteria comitans nervi peronei communis." Manno ('06 a), p. 275.

⁵ Weber described the a. articularis propria capituli fibulae in the following terms ('42, p. 207): "Sie ist etwa $\frac{1}{2}$ " dick, entspringt am Ende der A. poplitea, und tritt quer nach aussen zum Köpfchen des Wadenbeins, und verläuft von dem äussern Kopf des M. soleus und dem Ursprungstheil des M. peroneus longus und Extensor digitorum communis longus bedekt," u. s. w.

forms the function of transmitting blood to the embryonic a. nervi peronaei profundi, which becomes the distal part of the adult a. tibialis anterior. At about the stage of 20 mm., this function having been assumed by the embryonic artery that becomes the proximal part of the definitive anterior tibial artery, the a. nervi peronaei communis is described as being reduced in size, but as persisting in the form of the a. genu inferior lateralis, the a. recurrens tibialis anterior and the anastomosis between them.⁶

There is no difficulty in understanding how an embryonic artery that follows the course of the embryonic common peronaeal nerve, as does the a. nervi peronaei communis of DeVriese, might persist in adult life as the a. comitans nervi peronaei communis. It would seem impossible, however, that such an artery should become transformed into the a. genu inferior lateralis and the a. recurrens tibialis anterior of the normal adult limb.⁶ The anatomical considerations militating against the acceptance of such an identification have been referred to in connection with one of the arteries described by Manno as an example of the a. comitans nervi peronaei communis.

DEVELOPMENT

That an a. comitans nervi peronaei communis occurs in some embryonic limbs there cannot be the slightest doubt, for the literature contains incontestable evidence of the existence of such an artery in the adult. The question, however, as to whether the artery is constantly present, either in the adult or in the embryo, is one which such personal experience as I have had would lead me to answer in the negative. As far as the adult limb is concerned, my information has been derived solely from the ordinary run of dissecting-room material, and in such material I have encountered the artery very rarely. This experience, however, is exactly what I should expect from the

⁶ "La partie proximale de l'art. nervi peronaei s'atrophie mais persiste chez l'adulte comme arter. recurrens tibialis anterior et plus haute, arteria genu inferior lateralis; l'arteria nervi peronaei profundi est l'arteria tibialis anterior de l'anatomie humaine." DeVriese ('02), p. 695.

result of an examination of a good deal of embryonic material, which has been mainly negative in character. Although I have examined the popliteal and sural arteries of many of the more fully developed embryos belonging to the collection of the Carnegie Institution, and the deep popliteal artery of many of those in earlier stages of development, I have not been able to find a branch of any of these arteries that follows the course of the *nervus peronaeus communis*. A small *a. comitans* may be overlooked occasionally perhaps in examining serial sections, but I am quite certain that no such branch has been present in any of the twelve tolerably well-preserved embryonic human lower limbs of which I have reconstructed all the arteries. I know of only two embryonic arteries that follow any part of the course of the common peronaeal nerve; one of these was found in the right lower extremity of an embryo of 15 mm. (no. 350 of the Carnegie Institute Collection), and the other in the left lower extremity of one of 12 mm. (no. 3 of the Cornell Collection).⁷ All the arteries of both lower limbs of these two embryos were reconstructed; the vessel under consideration occurs in one limb only.

The common peronaeal artery of the 15-mm. embryo proves to be nothing more than a large branch of the anterior tibial recurrent that accompanies the common peronaeal nerve as far as the upper border of the lateral condyle of the femur. It is not sufficiently long to reach the *a. poplitea profunda* and seems to have very little in common with the adult *a. comitans nervi peronaei communis*. In all probability it would have figured in adult life as an unusually large branch of the anterior tibial recurrent artery. The common peronaeal artery of the 12-mm. Cornell embryo is very much more suggestive, although in the present state of our knowledge the nature of its future course of development is a matter of pure speculation. In view, however, of the position occupied by the ischiadic and common peronaeal

⁷ I am greatly indebted to Prof. C. R. Stockard, of Cornell Medical School, New York, N. Y., and to Prof. George L. Streeter, of the Carnegie Laboratory of Embryology, Baltimore, Md., for their courtesy in allowing me to use these embryos for study.

arteries with respect to the tibial and common peroneal nerves, it seems not unlikely that the vessel in question may prove to be an example of the embryonic precursor of the adult artery described by Salvi.

In the 12-mm. embryo in which this vessel occurs, the tibial and common peroneal nerves, which in earlier stages of development diverge sharply from one another as they arise from the sacral plexus, run a parallel course through the proximal third of the thigh, and the ischiadic artery occupies the narrow interval between them. The artery then follows the lateral aspect of the tibial nerve in the usual manner, nearly as far as the level of the knee-joint. Just as the tibial and peroneal nerves of the left limb are beginning to diverge, which they do at a progressively increasing angle, the ischiadic artery gives origin to a common peroneal branch. The latter is an artery of rather large caliber, which, having followed the medial aspect of the common peroneal nerve for some distance, ends abruptly or possibly becomes collapsed at about the junction of the middle and distal thirds of the thigh. It gives origin to a few small branches that enter into the formation of a plexus near the anterior aspect of the ischiadic vein, and to a larger branch that runs for a short distance on the anterior surface of the common peroneal nerve. Since this particular common peroneal artery does not enter the anterior crural region, it cannot be regarded as the precursor of a fully developed *a. comitans nervi peronei communis*, unless it be assumed that it would have done so if the embryo had not perished. The actual length of the artery in the earlier stages of development is not a matter of great importance in the present connection, for Salvi refers to imperfectly developed examples of the adult artery. No representations of imperfectly developed examples of the artery are presented by that author, but it is probable that such a vessel is exemplified in the lateral branch of the popliteal of Barkow's plate 57, figure 1 ('68) and in the lateral branch of the sural artery illustrated in figure 99 of Henle's *Gefässlehre*.

Manno's case, in which the presence of the *a. comitans nervi peronei communis* is associated with persistence of the ischiadic artery in adult life, is most instructive in suggesting the means

by which a common peroneal artery originally taking origin from the a. ischiadica might figure in adult life as a branch of the popliteal artery. The relations illustrated in Manno's figure 1 do not differ materially from those encountered usually in embryos of 18 mm., except that the tibial and common peroneal nerves run a parallel course nearly as far distally as the superior angle of the popliteal fossa as they do normally in the adult. The ischiadic artery occupies the narrow interval between the two nerves, and the a. comitans nervi peronei communis takes origin from the a. poplitea. The figure illustrates rather strikingly the influence that might be exerted by the gradual approximation of the tibial and common peroneal nerves of the 12-mm. embryo under consideration, upon the definitive mutual relation of the two arteries contained in the angular interval between them. The gradual obliteration of the space between the two nerves would almost certainly have initiated a process of progressive union between the ischiadic artery and its common peroneal branch which would have produced an effect of distal migration of the site of origin of the latter vessel. If this process had extended beyond the region of the hiatus tendineus, the origin of the common peroneal branch would automatically have been transferred from the ischiadic to the part of the deep popliteal artery that forms the proximal part of the adult a. poplitea. If a common peroneal branch of the ischiadic artery such as that described above should have failed to undergo the migration here suggested before the stage of 19 mm., it might be expected to disappear altogether to become converted into a common peroneal branch of one of the adult aa. perforantes.

It is scarcely safe to assume that the a. comitans nervi peronei communis of Manno's case arose at first from the a. ischiadica and became transferred to the popliteal artery in the manner suggested or that the definitive site of origin of that vessel is usually determined by a process of embryonic migration. It seems not improbable, however, that migration may be one of the regular events in the developmental history of the adult artery described by Salvi, and until such a branch of the popliteal artery, or of the part of the deep popliteal artery that enters

into the composition of that vessel, shall have been found in the embryo, it would be unwise to disregard the possible significance of a common peroneal branch of the ischiadic artery.

I have been greatly interested in the *a. nervi peronei communis* described by DeVriese, both in connection with its apparent similarity to the vessel described here as a somewhat doubtful example of the embryonic precursor of the *a. comitans nervi peronei communis* and on account of the important part it is said to play in the development of the *a. tibialis anterior* and its anterior recurrent branch, and the *a. genu inferior lateralis*. I do not believe that a common peroneal branch of the ischiadic artery, which appears so rarely in the embryonic limb, can take part in the normal development of the adult arteries just mentioned.⁸ On this account I have examined the evidence presented by DeVriese in order to ascertain, if possible, whether the *a. nervi peronei communis* was actually present in any of the embryonic limbs examined by that author; the result of this examination has not been at all convincing.

Sections from the lower extremities of embryos of 10, 13, 16, 20 and 27 mm., respectively, are represented. No artery accompanies the part of the common peroneal nerve shown in figure 23, taken from an embryo of 27 mm., and no sections from suitable levels of the embryos of 16 and 20 mm. have been reproduced. The figures representing sections of the 10- and 13-mm. embryos seem to bear unmistakable evidence of the inadequacy of the preservation of the material from which they were drawn. The ischiadic artery, for instance, seems to have been unrecognizable in the section reproduced as figure 15. It should, no doubt, have occupied the interval between the tibial and common peroneal nerves, as it is shown to do in figures 20 and 21, which represent sections of 16- and 20-mm. embryos, respectively. The wide lacuna around the tibial and common peroneal nerves depicted in figure 15, which is labeled '*a. ischiadica*,' appears to be an

⁸ The manner in which I believe the *a. tibialis anterior* and the *a. recurrens tibialis anterior* to be developed is described on pages 92 and 93 of the "Development of the arteries of the human lower extremity," *Am. Jour. Anat.*, vol. 25. The *a. genu inferior lateralis* is present in embryos of about 23 mm.

artifact, and the same may be said of the lacuna shown to surround the common peroneal nerve of figure 16.⁹

The author's conception of the relation borne by the 'lacune vasculo-nerveuse' of inadequately fixed human material to the vascular plexus, described as ramifying upon all the nerves of the embryonic mammalian extremities, is stated clearly on pages 675 and 676. The opinion that the dimensions of perineural lacunae of this kind are proportional to the number and size of the vessels destroyed in their production (p. 676) is one, however, that may be regarded as open to question. I have been unable to detect the presence of perineural lacunae in perfectly fixed mammalian embryonic material, human or otherwise, and, to judge from the appearance presented by figures 20 to 23, they do not seem to have occurred in the older embryos used by DeVriese. Owing to the nature of the circumstances under which mammalian embryos are obtained for fixation, perineural spaces are to be observed more commonly around the nerves appearing in serial sections of human embryos than around those seen in sections of other embryonic mammals. Considered in conjunction with other evidences of maceration of the tissues prior to fixation, the dimensions of such lacunae seem to me to afford a fair general indication of the degree of unreliability of any material in which they occur for the study of arterial development.

It becomes clear upon studying DeVriese's figures, that two classes of spaces have been labeled as arteries, namely, sections through arteries recognizable as such and sections through perineural lacunae. Under the circumstances referred to in the preceding paragraph, I see no reason for accepting the latter as equivalent to perineural arterial plexuses, even in the conventional sense noted on page 676. I find also, upon leaving out of consideration the perineural spaces that have been labeled as arteries, that all the arteries encountered usually in the developmental stages illustrated are still represented in the figures. I find it extremely difficult on this account to escape the conclusion

⁹ Both Elze ('07) and Evans ('12) have questioned the adequacy of the preservation of the material used by DeVriese.

that the a. nervi peronaei profundi of figure 16 and the a. nervi peronaei superficialis of figure 17 are not arteries, either in being or in process of development. Having been led into a somewhat similar error of interpretation through the study of poorly preserved material, I venture to offer this criticism of another's work in a spirit of sympathetic appreciation of the difficulties involved.

CONCLUSIONS

1. That the literature relating to the arteria comitans nervi peronaei communis is apt to convey an erroneous impression as to the completeness of our knowledge both of the adult vessel and of its embryonic precursor.

2. That the comitans nervi peronaei communis is not to be found in all adults, as has been stated by Salvi.

3. That the a. nervi peronaei communis described by DeVriese does not take part in the normal development of the a. genu inferior lateralis, the a. recurrens tibialis anterior, and the a. tibialis anterior, as stated by that author, although a branch of the ischiadic artery to which this name might be given is known to accompany the common peronaeal nerve of some embryonic limbs.

4. That it is doubtful whether an unquestionable example of the embryonic precursor of the a. comitans nervi peronaei communis has ever been described, either in this paper or elsewhere.

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Resumen por el autor, Wilbert A. Clemens.

Un caso de hermafroditismo completo en la rana toro (*Rana catesbiana*).

El ejemplar objeto de este trabajo fué descubierto durante una disección en los trabajos de laboratorio llevados a cabo por los alumnos. Presenta los siguientes caracteres masculinos: Un testículo izquierdo que contiene espermatozoides (con toda probabilidad existía también originariamente un testículo en el lado derecho); vesículas seminales pares, sacos vocales pares, y callos sexuales en ambos pulgares. También presentaba los siguientes caracteres femeninos: Ovarios pares que contienen óvulos y oviductos en ambos lados del cuerpo. Parece indudable que esta rana poseía órganos reproductores masculinos y femeninos completos y funcionales.

Translation by José F. Nonidez
Cornell Medical College, New York

A CASE OF COMPLETE HERMAPHRODITISM IN A BULLFROG (*RANA CATESBIANA*)

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ONE FIGURE

During the spring of 1919, the writer discovered a case of complete hermaphroditism in a bullfrog (*Rana catesbiana* Shaw). The specimen was under dissection in a laboratory class and unfortunately was not noticed until after some of the viscera had been removed. A considerable number of cases of various degrees of hermaphroditism have been reported in European Anura, but apparently the only previously described case in American Anura is that of an individual of *Bufo lentiginosus* (King, '10).¹ The case here reported is therefore remarkable when one considers the number of frogs dissected annually in laboratory classes.

The origin of the specimen is not known definitely, but the supply of frogs for that year and for the previous five years came from Keene, Ontario, from a dealer who maintained a frog pond there, and it is likely that the frog was produced at that place. The frogs were usually received about the middle of September.

The specimen is an adult of large size, as is shown by the following measurements: length from anus to tip of snout, 14.5 cm.; width of head at posterior angles of jaws, 5.8 cm.; width of tympanic membrane, 1.7 cm.; length of extended posterior right leg from anus to tip of fourth digit, 20.4 cm.

Externally there is nothing abnormal in the appearance of the specimen except that the shape of the body somewhat suggests

¹ King, Helen D. 1910 Some anomalies in the genital organs of *Bufo lentiginosus* and their probable significance. *Am. Jour. Anat.*, vol. 10, p. 159.

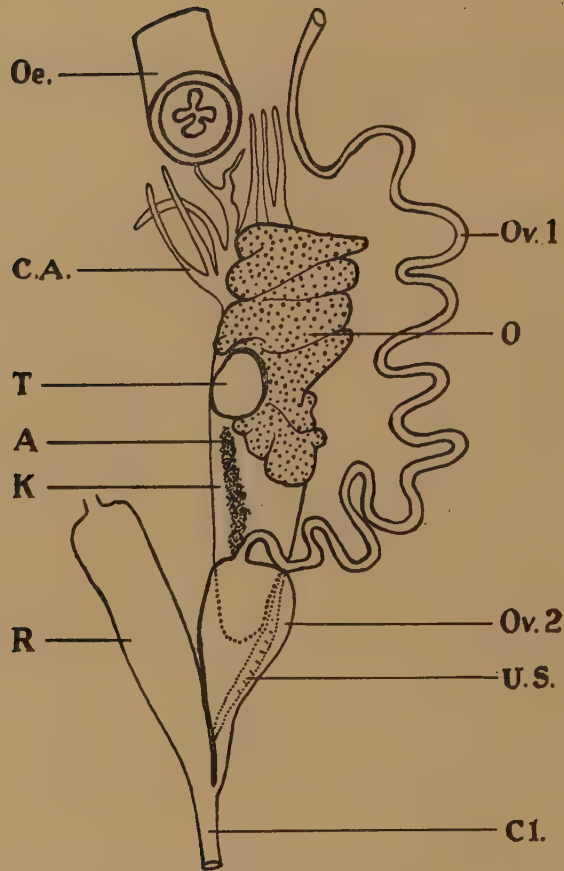


Fig. 1 Semidiagrammatic drawing of a ventral view of the reproductive organs of left side of an hermaphroditic bullfrog (*Rana catesbiana* Shaw). Natural size. A, adrenal glands; Cl, cloaca; C.A., corpora adiposa; K, kidney; O, ovary; Oe, oesophagus; Ov₁, convoluted portion of oviduct; Ov₂, enlarged posterior portion of oviduct with posterior end of kidney and ureter and seminal vesicle dorsal to it; R, rectum; T, testis; U.S., ureter and seminal vesicle.

that of a female, while the presence of pads on the thumbs indicates maleness. The vocal sacs and their openings into the mouth cavity are well developed.

Internally all the organs and organ systems were normally developed except the reproductive system. The student removed the respiratory system, the major portion of the digestive system, the heart and many blood vessels, the right ovary, and a portion of the right oviduct in the course of the dissection. His drawings, however, of these systems did not show anything abnormal. On the left side there is a typical ovary, fan shaped when spread out, having a length along the outer margin of approximately 10 cm. and a width of 2 cm. The ova can be seen with the naked eye and average about 0.5 mm. in diameter, although one was observed which had a diameter of 1.3 mm. and showed light and dark poles. These ova are thus about the normal size for this species for September, when the frog was probably killed. In the lower part of the ovary is embedded a testis approximately 6×8 mm. It contains fully developed spermatozoa. Vasa efferentia cannot be accurately identified and possibly have been destroyed. The student stated that there had been an ovary on the right side, but whether there was also a testis is not known. However, well-developed seminal vesicles are present on the ureters of both sides, which would seem to be good evidence for the presence of paired testes.

SUMMARY

Male characters

A left testis, containing spermatozoa (doubtless a right testis was also present originally).

Paired seminal vesicles.

Paired vocal sacs.

Paired thumb pads.

Female characters

Paired ovaries, containing eggs.

Paired oviducts.

There would seem to be no doubt, therefore, that this frog had complete and functional paired sets of both male and female reproductive organs.

Resumen por el autor, F. H. Swett.

Posición invertida de las vísceras en mónstruos dobles de trucha.

El autor describe brevemente en el presente trabajo quince embriones dobles de trucha con especial mención de la disposición de sus vísceras. En nueve casos la posición de las vísceras de ambos componentes es normal; en uno, la de A (el gemelo del lado derecho) está invertida; en dos, la posición de las vísceras de B (el gemelo del lado izquierdo) está invertida; y en tres casos B es normal y A presenta una posición visceral indeterminada.

Hay algunas indicaciones sobre la existencia de una correlación general entre el grado de duplicación y la presencia de una posición visceral invertida, pero la significación de dicha correlación es dudosa. Los gemelos de tipo parásito pueden presentar posición visceral invertida. Dicha posición puede presentarse en cualquiera de los gemelos, si bien en general aparece más a menudo en el del lado derecho (componente A).

Translation by José F. Nonidez
Cornell Medical College, New York

SITUS INVERSUS VISCERUM IN DOUBLE TROUT

F. H. SWETT

Osborn Zoological Laboratory, Yale University

SIX FIGURES

This investigation on situs viscerum was undertaken at the suggestion of Professor Harrison, to whom the writer gladly acknowledges his indebtedness for helpful criticism and advice. The material consists of a small collection of trout embryos (*Salmo fario*) taken by Prof. A. Petrunkevitch in Freiburg in 1901, a number of which show abnormalities of different kinds. The results are presented in the hope that they may prove of value in supplementing previous observations, particularly those of Morrill ('19), who made use of similar material. For this reason, then, very little attempt has been made to explain the extraordinary conditions of the viscera; they are merely described briefly and the relationship of the degree of external and internal doubling to the occurrence of situs inversus, in so far as it is disclosed by these animals, is shown.

From these trout embryos, fifteen which showed doubling to a greater or less degree were isolated. The specimens had been fixed in corrosive sublimate and preserved in 70 per cent alcohol, and it was found necessary to run them back to water before the yolk mass, which in each case united the two components, could be dissected off without damage to the underlying tissues. The region of fusion of the vertebral columns is taken as the criterion of the amount of external doubling, that of the intestinal tracts of internal doubling. The embryos show a fairly complete gradation in amount of doubling from almost total separation of the vertebral columns to a very close union as parasite and autosite. These terms are used to denote, respectively, the smaller and larger of the two unequal compo-

nents of the same monster. In every case one abdominal cavity is common to the two components, the ventral body walls being fused around a common yolk mass. In some cases there is such distortion of the viscera that it is almost impossible to decide whether the situs is normal or not. The asymmetry of the hearts could not be made out with certainty. The dissections were all made under the low power of the binocular and the drawings are from camera-lucida sketches of these dissections (magnification, $\times 8$.) The nomenclature adopted by Morrill ('19) for the components of the monsters is followed in each case i.e., designating as A the right twin (at the observer's left in a ventral view), and as B the left twin (right from the ventral aspect). The components are thus referred to in the descriptions, table, and figures.

MORPHOLOGICAL FINDINGS

The embryos are arranged in this section in the order of their degree of external doubling.

No. 1. The components of this monster are united by their ventral surfaces so that their median planes are almost coincident. Component A is slightly larger than B, showing a higher degree of development. The right eye of A is normal, the left has a rudimentary lens, as does the right eye of B, while the left eye of B is not visible externally. The vertebral columns of both components are separate to near the base of the tail, where the body musculature fuses more completely, forming a single tail. There are, however, two dorsal and two caudal fins. The abdominal cavity contains the viscera of the two components situated on diametrically opposite sides of the yolk mass. The intestinal tracts are separate until just before they open at the anus; during the passage from the abdominal cavity to this point they are closely associated side by side as parallel tubes.

The situs of the gut of A is normal, the stomach curving first slightly to the left, then making a wider curve to the right to pass over into the intestine which goes straight caudad to the anus. The liver lies on the right side of the intestinal canal.

The swim bladder, after its origin from the dorsum of the gut well toward the anterior end of the coelom, lies on the left of the canal. The curvatures of the gut of B are normal, as is also the liver, though this organ extends ventrally across the gut farther than normal, displacement being due probably to the pressure of the yolk mass. It sends a small pointed lobule cephalad on the right side of the stomach. The swim bladder of B lies on the right side of the gut immediately after its origin from the dorsal surface and continues in this position to its termination beneath the liver. This abnormal position may also have been due to the pressure of the yolk mass.

No. 2. The vertebral columns are separate to nearly the tip of the tail, the caudal fin is single. The head of A is slightly larger than that of B. The gut of each is normal, showing normal curvatures, and is single to a point slightly anterior to its exit from the body cavity. Here the intestines of the two components fuse. The liver of B is normal in size, shape, and position. That of A is rotated to a more transverse position than usual, otherwise it is normal. The swim bladders are both normal in position.

No. 3 (fig. 1). This specimen shows a typical case of situs inversus in the right twin (component A). The vertebral columns are fused posterior to the pelvic fins, the components are of nearly equal size, externally normal, and so placed that the dorsal fins lie almost in the same plane. The gut passes down the left and right sides of the body walls of components A and B, respectively, to a point about halfway between the pylorus and the exit from the body cavity, where the two fuse into one. Then the common intestine crosses to the ventral side of the coelom and passes back to the anus, where it opens to the outside. The shape of the two stomachs, livers, and swim bladders is normal, but these organs in component A are mirror images of those in component B. The liver of B lies on the right side of the stomach and ventral to the first part of the intestine, with its convex border toward the right; the greater curvature of the stomach is towards the left and is paralleled by the swim bladder. The stomach of A, on the other hand, has its greater

curvature toward the right, and the swim bladder is also on this side. The liver, with its convexity toward the left almost in contact with the corresponding part of the liver of B, is on the left side, its posterior end swinging to the right across the pylorus and the beginning of the intestine.

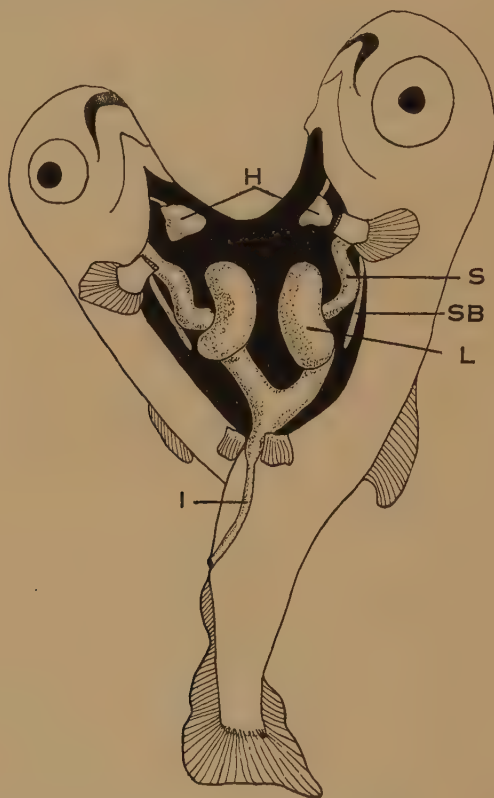


Fig. 1 Ventrolateral view, specimen no. 3. The situs viscerum B is normal, A is reversed. *H*, heart; *L*, liver; *SB*, swim bladder; *S*, stomach; *I*, intestine.

No. 4 (fig. 2 and 3). Situs inversus viscerum of the left twin is clearly shown in this embryo. The two components are of equal size and are placed in almost exact ventral apposition. It was found necessary to cut them apart to show the internal anatomy. The vertebral column of each is single to a point

slightly posterior to the dorsal fin, where the myomeres of the contiguous sides become fused. The adipose fins are separate, though one caudal fin is common to the two components.

The gut of component A is obviously in normal situs, though there is a considerable curvature of the cardiac end of the stomach

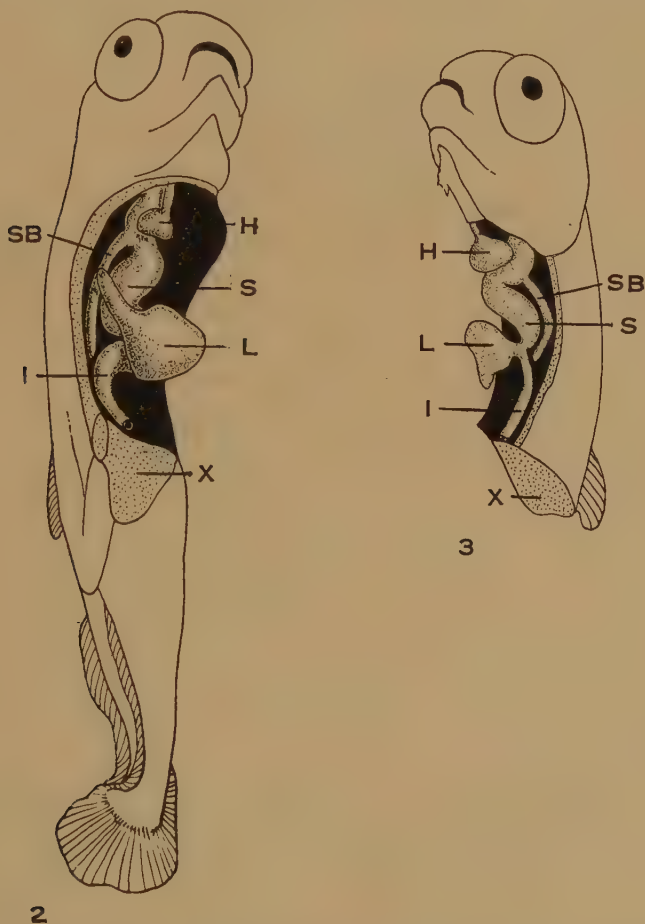


Fig. 2 Component B of specimen no. 4, ventrolateral view. X marks the point of separation from component A (fig. 3), other abbreviations as in fig. 1. This twin shows situs inversus.

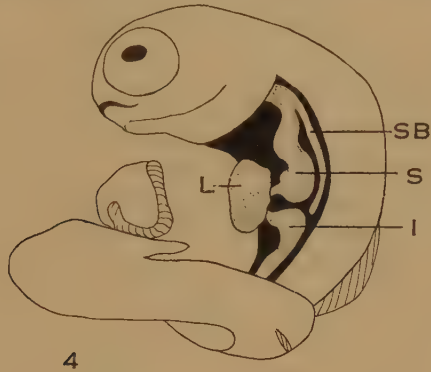
Fig. 3 Component A of specimen no. 4, ventrolateral view. Situs is normal. Abbreviations as in figs. 1 and 2.

ventrad and to the right caused by the pressure of the yolk mass upon it. The gut is single until just before its exit from the abdominal cavity, at which point the intestines of the two components fuse and pass to the anus as a single tube. The liver of component A, smaller than normal and somewhat wedge-shaped, occupies a ventral position a little posterior to and to the right of the middle of the common abdominal cavity. It is placed transversely with reference to the gut on the right side of the pylorus. The swim bladder of A is in its normal position at the left of and parallel to the stomach.

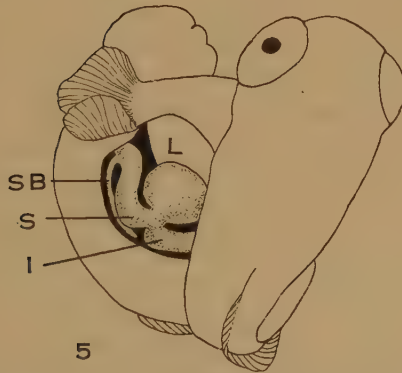
The stomach of component B, on entering the abdominal cavity, passes diagonally to the left for a short distance, then, just beyond the origin of the swim bladder, turns sharply to the right; it then curves to the left, gradually at first, then more abruptly beneath the liver, again curving sharply to the right at the pylorus to emerge as the intestine from beneath (dorsal to) the liver immediately caudad to the place where it disappeared. Thence it passes in a direct course to its point of fusion with that of component A. The liver, about the size and shape of that of component A, lies ventral to and covering the pyloric bend, its largest part extending some distance to the left of the gut toward the ventral wall of the common abdominal cavity. It sends an elongate, finger-like process cephalad and to the right over the ventral surface of the cardiac stomach. The swim bladder emerges from the gut on the right side and keeps this position with reference to the stomach and the first part of the intestine to a point near the lower margin of the liver, where it terminates.

No. 5 (figs. 4 and 5). This specimen also shows inversion of situs viscerum in component B. The vertebral columns are separate to a point just posterior to the dorsal fins, where they become fused. The caudal fin is doubled. Component B has an abnormal head, smaller than that of A and possessing no eyes and only a rudimentary mouth. Fusion of the intestines occurs just before exit from the abdominal cavity. The viscera of the autosite (component A) are in normal situs, only slight distortion due to crowding being noted.

The curvatures of the gut of the parasite (component B) are apparently reversed, the stomach first curving to the left and ventrally, then slightly dorsad and to the right. Just posterior to the origin of the swim bladder the greater curvature sweeps



4



5

Fig. 4 Left posterolateral view of specimen no. 5, showing arrangement of of the viscera in the autosite (component A). The situs viscerum is normal. Abbreviations as in fig. 1.

Fig. 5 Right posterolateral view of the same monster shown in fig. 4, showing the viscera of the parasite (component B). The situs viscerum is reversed. Abbreviations as in fig. 1.

posteriorly to the left and slightly ventrad, turning abruptly dorsad at the pylorus to meet the intestine. The liver is discoid in shape and lies to the left of the pylorus. The swim bladder lies dorsolateral to the stomach on the right side.

No. 6. Fusion of the vertebral columns takes place just posterior to the dorsal fins. Component A shows normal development and situs viscerum, but component B is much smaller, possessing no eyes or nares, and only a rudimentary mouth. Its viscera, however, are well developed, but show such great distortion that it is impossible to locate the primary curvatures with exactness. The liver of A, placed to the right of and ventral to the pylorus, is normal in position though slightly abnormal in shape. Its duct enters the gut posterior to the point where it is joined by the intestine of component B.

The gut of the parasite (B), on appearing in the abdominal cavity, passes transversely across it, then makes a sharp turn caudad and to the left, lying in contact with that of the autosite. At the pylorus it makes a slightly wider turn to the right and very shortly fuses with the pyloric stomach of component A. The liver of B lies to the left of the pylorus close to the dorsal wall, its duct enters the gut before the latter's fusion with that of component A. The swim bladders of both components lie in their normal positions with reference to the stomachs.

No. 7. Fusion of the vertebral columns takes place just posterior to the dorsal fins; more caudad there is a single body and tail. Component A is a very small parasite on component B, the head being only about half the normal size and possessing no eyes, nares, or mouth. The intestinal tracts of the two components are separate throughout, the intestine of the parasite being the more poorly developed and opening dorsal to that of the autosite into the single anus. The situs viscerum of both components is normal, the viscera of the parasite being much distorted anteriorly because of lack of space and considerably underdeveloped posterior to the pylorus.

No. 8. The heads of both components are of the same size and entirely separate to a point just posterior to the gill region. The vertebral columns are separate anterior to the dorsal fin, and both pairs of pectoral fins are present. The gut of each component presents curvatures indicating normal situs, though a considerable degree of distortion is shown, particularly in that of component B. The two fuse near the pyloric ends of the

stomachs. The liver appears as a single bilobed mass lying on (ventral to) the point of fusion of the gut of the two components. The larger lobe is somewhat quadrangular in shape, lying ventral to and to the right of the stomach of component A. The smaller, more anterior lobe of the liver mass extends anteriorly in the midline of the monster, filling the interval between the two stomachs. This anterior lobe apparently represents the liver of component B, and the posterior that of component A. The swim bladders were not found, having probably been destroyed in dissection.

No. 9. Fusion of the vertebral columns takes place just anterior to the dorsal fin (which is doubled). The head of each component is subnormal in size, though the two are approximately equal, that of A showing only a rudimentary lens on the left side, with no external trace of an eye on its right. Fusion of the two alimentary tracts occurs at the pylorus. The curvatures of the gut of both components are normal, but both livers and the swim bladder of B are displaced. The liver of A lies transversely across the ventral side of the first part of the common intestine; that of B, the smaller of the two, lies to the left of and caudad to the greater curvature of the stomach. Its duct, however, can be traced across the ventral surface of the gut to its normal point of communication with it. The swim bladder of A is in its normal position, while that of B appears on the right side of the stomach soon after its origin from the dorsum of the gut.

No. 10. The doubling of the vertebral column in this specimen persists posteriorly to a point midway between the tip of the midbrain and the anterior limit of the dorsal fin. The left and right pectoral fins of A and B, respectively, are coalesced to form an abnormal doubled structure, fused proximally, but distinguishable as two distally. The two heads are normal and of equal size. The cardiac ends of the stomachs of the two components rapidly converge on entering the abdominal cavity so that for the greater part of their length they lie side by side with a very narrow interval between them. Near the pylorus they fuse, and caudad to this point the intestinal tract is common to the two components. The curvatures of the gut indicate a

normal situs. The liver is compound, rather larger than a normal single organ, and is placed transversely across the posterior part of the abdominal cavity. It bears two small, obtuse lobules on the right border, overlapping ventrally the pyloric stomach of component A, and one smaller acute lobule extending cephalad between the stomachs. The swim bladder of component A is not demonstrable, that of B emerges from beneath the stomach to lie in the interval to the left of and paralleling the anterior lobe of the liver mass.

No. 11. The vertebral column is doubled anterior to a point midway between the anterior tip of the head and the dorsal fin. The head of component B is set at almost a right angle from the left side of the gill region of A, and in consequence does not extend so far anteriorly. The left eye of A and the right eye of B are lacking. The pectoral fins of the contiguous sides are fused and are smaller than a normal fin. The gut of component B makes a sharp turn caudad as it appears in the abdominal cavity, extending posteriorly to meet and fuse with that of component A in the pyloric region. From this point on the gut is single. The curvatures of both indicate a normal situs. The compound liver is an abnormally small organ placed slightly ventrad, caudad and to the right of the point of fusion of the digestive canals. The swim bladder of A passes posteriorly in the normal position at the left of the stomach, crossing it dorsally where the stomach makes the final bend before fusing with that of B, and extending still further posteriorly under cover of the liver. The position of the swim bladder of component B was not determined.

No. 12. The vertebral column is single posterior to a point midway between the anterior end of the head and the dorsal fin. The head of component B is smaller than that of A and lacks eyes. Fusion of the gut of the two components takes place at the region of the pyloric stomachs. The situs viscerum B is normal, though the liver is displaced to the left side of the abdominal cavity. The gut of component A presents curvatures of a type almost exactly mirror-imaging those of B. They are, however, not typical and the situs is not clear. The liver,

perhaps in part fused with that of B, shows only as a small mass situated between the two stomachs. The swim bladders were not found.

No. 13. Fusion of the bodies of the two components takes place immediately posterior to the gills. The head of B is normal, that of A is smaller and lacks the left eye. The viscera of the monster show considerable distortion, but the situs of component B is obviously normal. The stomach of component A extends caudad to fuse with that of B in the pyloric region, situs indeterminate. The liver is single and situated on the extreme right side of the abdominal cavity ventral and to the right of the pyloric region. The swim bladders were not visible.

No. 14. Only the head and three cervical segments are doubled, fusion taking place in the vertebral columns a very short distance posterior to the base of the skulls. Both heads are normal and equal in size and development. The stomachs of the two components lie very close together and fuse in the middle of the cardiac regions. The curvatures are apparently normal. The livers are fused and the mass of liver tissue lies ventral to the pyloric bend. The swim bladders are normal in size and position, that of A being hidden from view ventrally by the stomachs and pylorus.

No. 15 (fig. 6). The head of the parasite (component A) is hardly more than a bud from the right side of the anterior trunk region of the autosite, the proper vertebral column being very short. It is almost entirely devoid of recognizable organs, only small rudiments of gills being visible. The guts are fused in the region of the pyloric stomachs. That of the autosite presents normal curvatures, while the parasite shows them slightly reduced and apparently mirror-imaged. The stomach of A passes caudad with very gentle curves first to the left, then a longer one to the right, followed by a sharp turn toward the left to the point of fusion with that of B. A single liver mass (possibly compound) is present, situated on the right side of the abdominal cavity just lateral to the pylorus. It is about the size of a normal single organ. The swim bladders of both components are seen protruding from beneath the right side of the cardiac

stomach of component A, the one from B obviously crowded out of position by yolk, while that of A lies on the right side of the stomach throughout its course.

DISCUSSION

The asymmetry relations in the viscera of the double trout examined in this study bear out in general the view set forth by Morrill ('19) that the correlation between the amount of external doubling and the occurrence of situs inversus viscerum

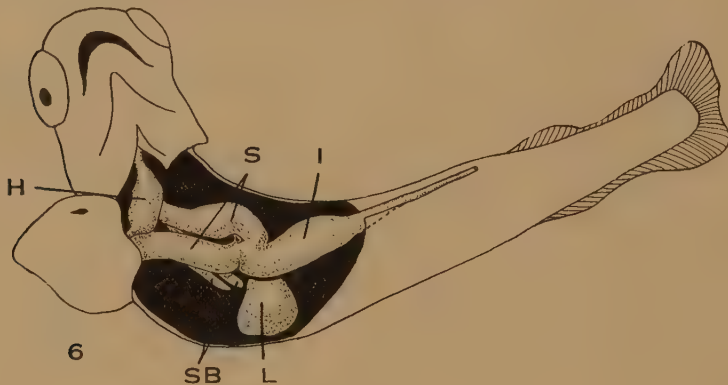


Fig. 6 Ventral view of specimen no. 15. Component A is of the parasite type, B the autosite. Situs B is normal. The gut of A mirror-images that of B. Inversion is not certain; the effect may be due to torsion. Abbreviations as in fig. 1.

is not at all precise. In table 1 the monsters are arranged according to their degree of external doubling. It will be seen that those whose vertebral columns are fused anterior to the dorsal fin and those which are nearly separate show at best only doubtful mirror-imaging. It is further noted, as was also found by Morrill, that not all the animals falling within the limits of doubling apparently most favorable for transposition of the viscera show this phenomenon. Thus there are indications that the reversal of asymmetry is not a necessary consequence of any condition of doubling, but may or may not occur, depending on some other factor capable of operating within these limits.

Column 5 of the table shows the order in which the specimens in this series appear when arranged according to the degree of internal doubling. This indicates a similar condition of correlation to that shown for external doubling, mirror-imaging only occurring when the digestive tracts are fused at some point be-

TABLE 1

Showing trout embryos arranged according to degree of external doubling

NUMBER	SITUS A	SITUS B	REGION OF FUSION VERTEBRAL COLUMN	ORDER OF INTER- NAL DOUB- LING	REGION OF FUSION OF GUT
1	Normal	Normal	Base of tail	2	Just anterior to anus
2	Normal	Normal		5	Between pylorus and exit from body cavity
3	Reversed	Normal		6	
4	Normal	Reversed		3	
5	Normal (autosite)	Reversed (parasite)		4	
6	Normal (autosite)	Normal (parasite)	Posterior to dorsal fins	7	Irreg. — pyloric stomach with intestine
7	Normal (parasite)	Normal (autosite)		1	Separate
8	Normal	Normal	Anterior to dorsal fins	12	Pyloric stomach
9	Normal	Normal		9	
10	Normal	Normal	Half way midbrain to dorsal fin	11	At pylorus
11	Normal	Normal	Half way snout to dorsal fin	8	
12	Indetermi- nate	Normal		14	Pyloric stomach
13	Indetermi- nate	Normal	Just posterior to gills	10	At pylorus
14	Normal	Normal	Ca. 3rd cervical segment	15	Midcardiac stom- ach
15	Reversed ? (parasite)	Normal (autosite)	Near base of skull	13	Pyloric stomach

tween the pylorus and the exit of the intestine from the abdominal cavity. The table clearly shows that there is no accurate correspondence between the degree of external and internal doubling. Spemann and Falkenberg ('19) find that situs inversus occurs in separate twins produced by constriction of the embryo at blastula or gastrula stage, and in the light of this it cannot be

said that mirror-imaging is in any way dependent on the degree of doubling, external or internal.

It is not in point to discuss in detail at this time the theories of the causes of situs inversus, and the reader is referred to Morrill ('19), pp. 275-81, for a concise review of the recent literature. Pressler ('11), working with Spemann's material, found situs inversus in larvae of *Rana esculenta* and *Bombinator*, which had had a portion of the medullary plate and the subjacent endoderm turned end for end in the neurula stage. Spemann ('18), working also with *Bombinator*, has shown that situs inversus can be obtained by inversion of a small bit of ectoderm and endoderm in the region of the future medullary plate at the end of gastrulation, but inversion of the ectoderm alone at the beginning of gastrulation has no effect. Spemann and Falkenberg ('19) found in the twins and double monsters of *Triton* produced by constricting the developing embryo at the gastrula, blastula, or even earlier stages, that approximately half of the right twins (corresponding to component A) showed situs inversus, the others being normal or of indeterminate situs. Spemann, in discussing the theories of the causal factors in situs inversus, considers a fundamental inherent bilateral asymmetry in the egg. He suggests as a possible interpretation of the occurrence of mirror-imaging in the right twin after a cut in the midline of the embryo, that the primary asymmetry existing in the right half of the gut anlage may be reversed in some such manner as is that of certain asymmetrical crystals after injury. He considers that this explanation, which was put forward by Přibram to account for reversal of asymmetry in a limb regenerated from a proximally directed wound surface, may be at least partially applicable to the phenomena which result in the establishment of situs inversus viscerum in the right twin. The possibility of reversing the microstructure of crystals by cutting seems to him highly suggestive in accounting for mirror-imaging in the gut, as inversion of the microstructure of the anlage of one twin would, in the course of subsequent normal development on this basis, produce situs inversus, just as normal development from a normal (not inverted) micro-

structure would result in normal situs. However, in view of the fact that constriction of an embryo of practically any age up to the completion of gastrulation may result in situs inversus viscerum of one of the twins so produced, Spemann considers it more probable that asymmetry reversal in the viscera is brought about as a direct effect of the injury rather than an inversion of the fundamental microstructure. Structural deficiencies are observed to preponderate on the operated side in such experimental animals and the bodies are often definitely bent toward this side. This shows there is a marked effect from the wound, and this influence may be sufficient to transpose the relations of the viscera without any molecular rearrangement in the cellular make-up. The exact manner in which these fundamental changes are brought about remains for future investigations to determine. Spemann suggests that breeding animals with situs inversus may help in clearing up this confusion.

Aside from the fundamental problem of the causes of symmetry reversal, another point deserves mention. Morrill ('19) states that it is component A which shows the mirror-imaging, if inversion is present, component B always being normal. Spemann, in the work mentioned above, describes an exceptional case showing mirror-imaging of the heart of the left twin; the situs of the gut of this twin was not clear. There was also mirror-imaging in the gut and heart of the right twin of this pair. He states that situs inversus almost never occurs in the left twin. In my cases no. 4 and no. 5 we have two more examples of inversion in the left twin (component B). Spemann, in explaining the inversion of situs cordis which he found in this left twin, states that it is probably due to an influence other than that of the operation, affecting the heart anlage from the outer side. In support of this view he cites cases of his own which show defects of the limbs, gills, etc., on the side of the body away from the cut, which have resulted from stimuli not connected with his operative procedure. He also brings up the experiments of Dareste ('77), who produced situs inversus ('l'hétérotaxie') in chicks by the application of excessive heat to the left side of the developing embryo, as corroborative of this point of view.

This work was later repeated by Warynski and Fol ('84) with similar results. It seems improbable that the extensive conditions of inversion found in the two double monsters no. 4 and no. 5 of this study could be due to the effect of some adverse external influence applied in just the right region and at precisely the right moment. As is shown by figs. 2, 3, 4 and 5, there are no unilateral structural abnormalities in either of these cases to be related to retarded or accelerated growth. Both components of no. 4 are externally normal, and though component B of no. 5 is of the parasite type, it is at least bilaterally symmetrical. Again, it is stated by Morrill that in his specimens of the auto-site-parasite type, where one component is considerably larger and better developed than the other, mirror-imaging never was found in the parasite, no matter on which side of the monster it was situated. Here it is necessary to call attention to specimen no. 5 (and possibly no. 15), in which it is the parasite which shows the inversion. Interpreting situs inversus according to the theory set forth by Spemann, it is difficult to see why the relative size of the twins should influence the occurrence of mirror-imaging. Indeed, if there were an effect, one might expect that the parasite, showing the poorer development, would more easily be affected than the autosite.

At present the ultimate cause of situs inversus is still hypothetical; the theories which have been advanced for its explanation are suggestive, but not conclusive. There are indications of a general correlation between the degree of doubling and the occurrence of mirror-imaging in the viscera, but since separate twins may also show situs inversus, the significance of any such correlation is very doubtful. Situs inversus may occur in either twin, though the large predominance of rights over lefts in this respect indicates that for some reason the two sides are not equally susceptible. Twins of the parasite type may show situs inversus. It is evident that more data and further experiments along these lines are necessary before a true solution of the problem can be reached.

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Resumen por la autora, Evelyn Holt.

Un proceso peculiar en el piso diencefálico del feto de ternera.

En el piso diencefálico del feto de ternera existe un proceso peculiar que aparece por primera vez en el estado de 48 mm. existiendo en todos los estados ulteriores. Este proceso se origina como una masa irregular que parte del límite posterior del quiasma en la línea media ventral, extendiéndose hacia abajo y posteriormente en el espacio subaracnoideo, para terminar de un modo difuso en el mesenquima de la región de la parte bucal de la hipófisis. El proceso mencionado no presenta nunca conexión alguna con otras estructuras y parece ser de naturaleza neurógica. Mientras que existe constantemente en el embrión de ternera, no ha podido hallarse en el cerdo, carnero o en el hombre.

Translation by José F. Nonidez
Cornell Medical College, New York

A PECULIAR PROCESS OF THE DIENCEPHALIC FLOOR IN THE FETAL CALF

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ONE FIGURE

From the diencephalon of the developing calf embryo is a curious ventral outflow of nervous tissue which was pointed out to me by Doctor Kingsbury and which hitherto has not been described. This structure does not appear in the early stages, but from the time of its appearance, in a 40-mm. embryo, was found present in all the specimens examined.

For this study series of older calf embryos were used. Specimens showing the head region cut in the sagittal plane included the following lengths: 30, 48, 58, 62, 80, 82, 94, 95, 110, 130, 167, 190, and 310 mm. In addition embryos of 33, 39, 40, and 50 mm: cut in the transverse plane were used for purposes of comparison. These embryos, the property of the Department of Histology and Embryology of the Cornell University Medical College at Ithaca, are cut and mounted serially, and stained with hematoxylin and eosin, hematoxylin and orange G, or hydrochloric acid carmine, with or without Lyons blue as a counterstain. All except the youngest specimens were decalcified before cutting.

The structure to be described first appears as a definite outflow in the 48-mm. stage, although there are indications of it at 40 mm. From the time of its appearance it is constant, becoming increasingly prominent in the older stages, and having certain characteristic relations throughout. These are shown in the accompanying figure, an untouched photograph of the hypophyseal region of a 110-mm. calf embryo. The photograph which represents a sagittal section near the midline is reproduced at a magnification of ten diameters. Arising as an irregular mass

from the posterior limit of the chiasma in the midventral line, this process of neural tissue extends downward and backward to terminate diffusely in the mesenchyme near the pars buccalis of the hypophysis. In all the specimens the process faded out in an indefinite brush-like manner, the ragged ends becoming lost in the mesenchyme, and never showing a connection with any other structure. It is interesting to note that even before

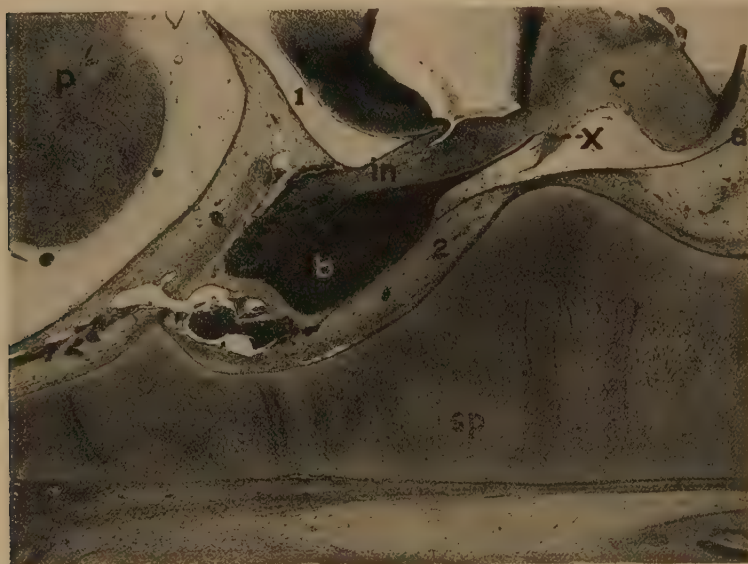


Fig. 1 Sagittal section near midline of hypophyseal region of 110-mm. calf embryo. $\times 13\frac{1}{2}$. *p*, pons; *c*, optic chiasma; *in*, infundibular process (pars nervosa of hypophysis); *b*, pars buccalis of hypophysis; *sp*, basilar plate (sphenoid); *x*, process herein described; *a*, developing arachnoid and dura (inner layer); *1*, subarachnoid space; *2*, mesenchyme of sella turcica.

the appearance of this structure as a definite outflow its point of origin is marked by the entrance of one or more small blood vessels into the neural tube. This fact may or may not have significance, but is rather noticeable because of its constancy. This outgrowth shows no signs of axis cylinders, is in no way connected with a ganglion, and appears to be neuroglial in character. Little, however, can be said concerning its actual struc-

ture, as it was not studied on material stained especially for nervous tissue.

Any description of the process must include a brief mention of the developing meninges and the hypophysis, to which structures it bears an intimate relation. Weed ('15) described the developing brain membranes of the pig, and his findings apply to the calf also. A mesenchymal condensation directly around the neural tube represents pia, while outside this the mesenchyme takes on the character of a loose meshwork, the spaces enlarging and the arachnoid trabeculae appearing as irregular condensations. Beyond the arachnoid trabeculae is a condensation which represents the arachnoid membrane and the inner layer of the dura. In the pig (Weed, '15) the line of separation between these two appears at 50 mm. In the calf the separation process is evident by 48 mm., but progresses rather slowly. The processes of mesenchymal arrangement and condensation which result in the formation of the meninges are well advanced before the first appearance of the outgrowth, and locating the outgrowth in terms of the membranes we may say that it is always confined to the subarachnoid area, never extending beyond the condensation which represents arachnoid and dura. As is well known, these membranes form a collar, the diaphragma sellae, about the neck of the hypophysis. This collar is pierced by the infundibular stalk and the pars tuberalis. The neural process confined to the subarachnoid space and directed toward the hypophysis suggests the possibility of a close, perhaps a functional, relation between the outflow and the gland. The best indication of this would be an actual contact between the two structures. Such a contact could never be observed. At its origin, however, the outflow bears a definite spatial relation to the pars tuberalis of the hypophysis. This tongue-like process (to use Herring's term) extending forward over the surface of the tuber cinereum ('postchiasmatic eminence'—Tilney, '15) stops immediately before it reaches the outgrowth, being separated from and bound to that structure by its own basement membrane, which is continuous with the pia, but which appears somewhat thicker and more compact than the typical developing

pia. The nasal horns of the tuberal processes (Atwell, '18) have fused across the midline before the appearance of the outflow.

Curiously enough, although a number of investigators (Herring, '08; Trautmann, '09; Wulzen, '14) have studied the hypophysis and hypophyseal region in the calf, no one has described this structure. Nor has it, so far as could be determined, been described in any other form. It is, as has been said, constant in all the calf embryos examined from the 40-mm. stage to and including a specimen 310 mm. in length. No embryos older than this were examined. The process is not mentioned by Tilney ('15) in his study of the comparative morphology of the diencephalic floor, and could not be found in pig, sheep, or human embryos. The pig embryos belonging to the Department of Histology and Embryology include lengths up to 54 mm., while the sheep embryos, kindly lent by Professor Gage, include lengths up to 44.5 mm. Because of the possibility that the structure might appear in older embryos than those examined, the diencephalic region of a 90-mm. lamb was studied. No sign of the nervous process was present. This nervous process is present in the calf, absent in the pig, sheep, and human, and undetermined in other forms.

While no theory is offered to explain this process, it is believed that the explanation will be found, not in the study of it alone, but in its relation to surrounding structures, especially the hypophysis cerebri, the mesenchyme, and the developing meninges. It may represent a portion of the brain wall which became fixed—possibly by a developing blood vessel—and was subsequently drawn out into an irregular process by the growth of surrounding parts, or it may indicate some primitive, little known, condition of development. Why it should exist in the calf, and apparently in the calf only, remains a puzzle. It seems to have no function, no homologue, no obvious reason for existing, and yet because it is constant in this form there must be some underlying cause, some point wherein the development of the diencephalic or hypophyseal region in the calf differs from the condition in other forms. This can be determined

only with a fuller understanding of the development of this region.

In conclusion, I should like to thank Doctor Kingsbury for the help which he has so generously given.

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Resumen por la autora, Evelyn Holt.

Ausencia de la parte bucal de la hipófisis en un embrión de cerdo de 40 mm.

El objeto del presente trabajo es la descripción de un embrión de cerdo de 40 mm., el cual aunque parecía normal en todos los demás rasgos, no poseía la parte bucal de la hipofisis. Mientras que dicha parte bucal falta, la parte neural está bien desarrollada, estando reunida con el cerebro, y presentando posición, tamaño y estructura normales. Está rodeada por una condensación de mesenquima que es continua con la pia y se parece a una membrana basal, excepto cerca del ápice del proceso, donde la diferencia entre los tejidos epitelial y neural se pierde por completo. La tiroides, suprarrenales y gonadas aparecen normales cuando se las compara con embriones de cerdo de 38 y 42 mm., respectivamente. Como no se ha descrito ningún caso semejante, esta anormalidad debe considerarse como rara. Tiende a rechazar la teoría que atribuye el desarrollo del proceso infundibular a la presión ejercida por la bolsa de Rathke sobre la vesícula cerebral anterior.

Translation by José F. Nonidez
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ABSENCE OF THE PARS BUCCALIS OF THE HYPOPHYSIS IN A 40-MM. PIG

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TWO FIGURES

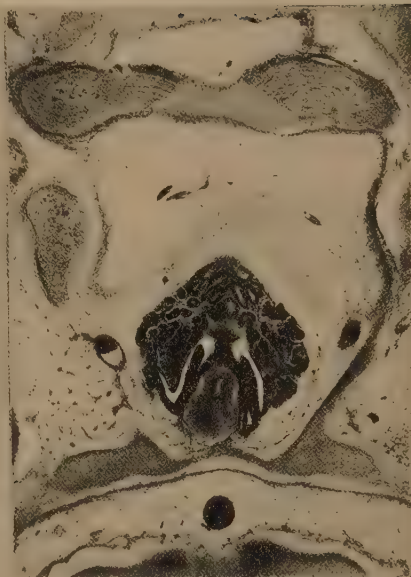
Belonging to the Department of Histology and Embryology at the Cornell University Medical College in Ithaca is a 40-mm. pig embryo, remarkable for the fact that, while the infundibulum is well developed, the oral portion of the hypophysis is wholly absent; in other respects the embryo appears normal. Because no similar case has been recorded and because this abnormality may help to make clear the normal development of the hypophysis, a brief description of the specimen is here given.

The embryo (series 148) is well preserved, cut transversely in sections 10 μ thick, and stained with hematoxylin and eosin. For purposes of comparison, there were used two pig embryos of 38 and 42 mm., respectively. These were also cut in the transverse plane. Photographs at a magnification of twenty-two diameters were made of the hypophyseal region of the 40 and 42-mm. pigs, and are here reproduced, side by side, as figures 1 and 1a, 2 and 2a, because it is believed that the untouched photographs present the true condition more accurately than line drawings or verbal descriptions. Owing to the inevitable slight difference in the plane of section, the levels do not correspond exactly.

In the 40-mm. pig the pars buccalis of the hypophysis is wholly absent, there being no trace of it in the usual position about the pars nervosa—where at this stage it should be a conspicuous feature—or in the roof of the pharynx, or along the route of development, where a craniopharyngeal canal sometimes exists. The pars neuralis, on the other hand, is well developed, connected with the brain, and normal in position, extent, and



1



1a



2



2a

structure. It is tubular, extends downwards and backwards from the diencephalic floor, and is made up of two zones, a medulla consisting of several layers of closely packed cells, and a cortex made up of fibers and cells in looser arrangement. This point, the normal appearance of the pars nervosa, is of special interest in connection with the work of Smith ('20), who removed the anlage of the epithelial hypophysis (pars buccalis) from frog larvae of 3.5 to 4 mm. in length.¹ On the basis of his work, Smith says (p. 78): "It thus seems clear that in the absence of a nearly normal epithelial component, the neural hypophysis can not undergo its normal development nor attain its typical size or shape." He found the pars nervosa in the operated tadpoles to be smaller than normal, asymmetrical in

Fig. 1 Section of the hypophyseal region of a 40-mm. pig embryo, showing complete absence of the pars buccalis.

Fig. 1-a Comparable section of a (normal) 42-mm. pig embryo.

In the mesenchyme of the sella turcica appears the pars nervosa, alone in figure 1, nearly surrounded by pars buccalis in figure 1-a. On either side of the hypophysis is the corresponding internal carotid artery. Anteriorly (above) the optic chiasma may be seen, while posteriorly (below) is a part of the cartilage of the sella turcica.

Fig. 2 Section of 40-mm. pig showing infundibular stalk connected with the brain.

Fig. 2-a Comparable section of 42-mm. pig.

From before backward the following parts of the brain appear, optic chiasma, postchiasmatic eminence (medial eminence of tuber cinereum), pars nervosa of hypophysis, and, behind the sella turcica, pons. The fact that in figure 2 the chiasma and postchiasmatic eminence appear continuous, while in figure 2-a they are separated by mesenchyme, is due to a slight difference in the plane of section. In figure 2 the pars buccalis is lacking and the pars nervosa is directly related to the mesenchyme, while in figure 2-a the pars buccalis is present appearing on either side of the infundibular process and extending forward (pars tuberalis) over the surface of the tuber cinereum. The internal carotid arteries appear as before.

¹ Smith classified the results of the operation as, 1) disturbances of pigmentation; 2) changes in the rate of growth, and, 3) endocrine disturbances. The first of these does not apply to the pig and will not be considered here. The second is difficult to determine from one specimen, especially as we have not the litter mates to use as a basis of comparison. In point of differentiation, however, the embryo is comparable to other pigs of the same length. The third point, endocrine disturbances apart from the pars nervosa of the hypophysis, will be considered a little later.

form, and atypical in position. As the oral component was removed before the formation of the primitive infundibulum and without injury to the neural tube, these changes were not considered traumatic, due to the operation. They might be explained as due to one of two factors or to these two acting conjointly. They might be an expression of a general endocrine disturbance due to hormone deficiency, atrophic or non-developmental changes comparable with the marked peculiarities noted by Smith (pp. 83 to 97) in the thyroid and adrenals; or they might be due to the lack of a mechanical stimulus normally derived from the pars buccalis. While he does not deny the possibility of the former factor, Smith states quite plainly (p. 83) that "the neural lobe and pituitary floor are dependent upon the association with the epithelial hypophysis for their full development." The pars nervosa of this pig embryo, being normal in position, size, and shape, does not support the theory that the association of a normal pars buccalis is necessary for the development of the infundibulum. It is probable, however, that the presence of the whole gland would be necessary for the complete development and normal functioning of the pars nervosa. Here we encounter a difficulty, as the exact functional processes of the gland and the relation of the two parts are little understood. That the relation is a close one seems evident on histological grounds. Atwell ('18, pp. 303, 304, fig. 21) states that in sixteen-day rabbit embryos there are definite contacts between the two portions of the hypophysis, these contacts indicating outgrowth of one or the other part. These may also be observed in pig and calf embryos. Herring ('08 a, p. 149) says that in adult cats epithelial cells may be demonstrated in the infundibular process, these cells having migrated from the pars buccalis. In that these contacts and ingrowths obviously cannot exist in the absence of the pars buccalis, the pars nervosa cannot reach full development alone; but that in the absence of the pars buccalis the infundibular stalk and process not only may appear, but may appear normal, in the mammal at least, is demonstrated by this embryo.

The infundibular process is surrounded by a mesenchymal condensation which is continuous with the pia and resembles a basement membrane except near the tip of the process where the line of demarcation between the two tissues becomes lost and there seems to be a confluence of neural and mesenchymal tissue. Here the nervous tissue appears to end in an irregular scalloped manner, while the mesenchyme filling in the irregularities (fig. 2) has the appearance of actually invading the nervous process. This is probably normal, as the same thing was observed in sagittal sections of 35- and 54-mm. pigs. Atwell ('18) states that in the rabbit portions of the original basement membrane may be seen between the cortical and medullary zones of the infundibulum. It was perhaps this appearance which led Müller and Mihalkovics ('75) to believe that mesenchymal cells replace the proper nervous tissue of the infundibulum, converting it into a connective-tissue appendage of the brain. Herring ('08) showed that connective tissue is never present in the infundibulum to a considerable extent, the fibers which were described as connective tissue being ependymal or neuroglial in character. The condensation which represents the arachnoid and inner layer of the dura appears to surround the neck of the infundibulum as it would were the whole gland present.

Upon careful examination, the development of other parts appeared normal, and that the embryo was alive and growing is proved by the presence of mitotic figures. The mesenchyme of the sella turcica shows evidences of erythrocyte formation and does not differ appreciably from the mesenchyme of this region in a normal embryo. The notochord ends, as in the case of the 38- and 42-mm. pigs, in the cartilaginous sphenoid. How it ended in an earlier stage when, in the pig, it would normally be attached to the wall of Rathke's pocket, we have, unfortunately, no means of determining.

Because of the emphasis placed by various workers on the relation between the hypophysis and the other ductless glands, the thyroid, gonads, and suprarenals of the three pigs were compared. The results of this comparison were negative. In

the 40-mm. pig the thyroid was slightly larger than in either of the others, but this difference did not seem sufficient to be connected in any way with the non-development of the hypophysis, especially as the gland was a trifle larger in the 38- than in the 42-mm. pig. The structure of the gland was similar in all cases, a well-vascularized cord- or platework of cuboidal cells with deeply staining nuclei. Colloid was not yet present. The ovaries of the 40- and 42-mm. pigs were similar in state of development. The 38-mm. pig was a male. The suprarenals seemed to be entirely normal. These observations differ from the findings of Smith ('20), but the difference may well be due to the fact that the pig develops in utero and receives pituitary secretion from the maternal organism. At any rate, this embryo offers no evidence of a primary relation between the endocrine organs.

While there are numerous descriptions of hypophyseal tumors and accessory glands, such as the pharyngeal hypophysis situated in the roof of the pharynx (Cushing, '12, and Schwalbe, '09), it has been impossible to find the record of a single case of absence of either lobe. As the pars buccalis is considered essential to life, an animal presumably could not live after birth with this portion of the gland congenitally absent. In this connection perhaps it should be noted that Smith's hypophysectomized tadpoles were never able to complete metamorphosis. As the literature contains no account of such an abnormality, whether in a fetus or in an animal after birth, it must be assumed that the defect is rare. Because neither part of the gland has been described as existing without the other, most workers have assumed the relation between neural and buccal parts to be fundamental.

Although there are several theories to account for the development and relations of the two parts of the gland, no one affords adequate explanation of this anomaly. His, Dursy and Müller, believed that the notochord exercised a mechanical influence in drawing out the infundibulum. Mihalkovics ('75), however, disproved this theory, and again Woerdemann ('14) pointed out that what the earlier workers had described as contacts

were not true contacts—that is, while the two parts may be close together they are always separated by a slight amount of connective tissue. Others, notably Reichert and His, noting the contact between the notochord and Rathke's pocket, held that the notochord served to draw out the oral portion of the hypophysis. Mihalkovics ('75), Woerdemann ('14), and Atwell ('18) disproved this theory, basing their conclusions on the following observations: The contact does not exist until after Rathke's pocket is formed; that is, the 'cause' follows the 'effect.' The contact is by no means constant; in some forms it appears at the superior pole of the pocket, in others at the inferior, while in the rabbit it is variable, present in some but not in all specimens.

Mihalkovics, observing that the oral evagination is the first to form and that it is closely related to the neural tube, attributed the formation of the infundibulum to the pressure exerted by Rathke's pocket. Or, as Herring ('08 a, p. 163), who adopted this theory, said, "The wall of the sac presses upon the base of the anterior brain vesicle, giving rise at its upper extremity to a fold which becomes the primitive infundibulum." This theory fails to explain the presence of the infundibulum in the absence of the pars buccalis, unless we assume that Rathke's pocket appeared, pressed upon the base of the brain to start an evagination, and then involuted quickly and completely. We have no basis for this assumption, and it would leave us with three problems instead of one—What caused the developing pocket to disappear? Why did not this factor cause some other abnormality? Why is there no trace of an oral evagination?

Wiley ('94, p. 287) believed the relation between neural and oral epithelia to be incidental, due perhaps to a tendency of contiguous embryonic tissues to fuse together. This seems improbable, because the relation between the two parts is close and constant, being marked from the earliest stages.

Minot ('92, pp. 573, 574) advanced another theory: "The ectoderm of the mouth over the hypophyseal area lies against and is apparently intimately soldered to the ectoderm of the brain, a point which has been generally overlooked, but which

seems to me of great importance. . . . It is probable that the cementing together over the hypophyseal area of the buccal and cerebral ectoderm is the mechanical condition causing the formation of the two diverticula." On the basis of this theory, we might explain the absence of the pars buccalis in the following manner: the neural and oral epithelia came together as usual (before the formation of the hypophyseal angle), then the growth of the embryo was disturbed so that Rathke's pocket failed to appear, the disturbance subsided, and the infundibulum, normally appearing somewhat later, developed as usual.

Perhaps it would be better to consider the development of the hypophysis in connection with the development of the rest of the head, paying particular attention to the mesoderm and the head bend. The cementing together of neural and oral epithelia without the intervention of mesenchyme is perhaps important as affording a fixed point. Then, Rathke's pocket would be due not to a simple evagination of oral ectoderm, but to the fact that at one point two layers of epithelia are held together, while at all other points they are separated by mesenchyme, the degree of separation increasing with the proliferation of the mesenchyme cells and the growth of the surface ectoderm. If at an early stage mesenchyme cells appeared between the oral epithelium and the wall of the brain, there would be no fixed point and consequently no Rathke's pocket. If this were so, the presence of the infundibulum would be determined by the growth of the neural tube in its relation to the surrounding mesoderm. The anomaly here described, while it renders improbable Mihalkovics' theory as to the mechanics of the development of the hypophysis, proves no other theory. It stands as an interesting and rare anomaly, an occurrence which must be explained by anyone who attempts to account for the development and relations of the two parts of the hypophysis cerebri.

To Doctor Kingsbury, who suggested this description and placed at my disposal the departmental material making it possible, I wish to express my thanks for his very generous help.

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ABSTRACT

THE VASCULARIZATION OF THE MEMBRANOUS LABYRINTH OF THE GUINEA-PIG, THE RABBIT, AND THE CAT.¹ By Denjiro Nabeya, M.D., Los Angeles, California. From the Anatomical Laboratory of the University of Chicago. Abstracted by George E. Shambaugh.

This is a careful piece of anatomical research undertaken on the suggestion of Doctor Shambaugh, who had previously worked out the blood supply of the labyrinth in the domestic pig, the sheep, and the calf. These investigations by Nabeya constitute a valuable addition to the study of comparative anatomy.

The methods employed were the celloidin casts as used by Eichler, Siebenmann and Shambaugh, supplemented by modification of the Spalteholz method of rendering anatomical preparations transparent.

In the arterial system for each of the three animals studied, the same general scheme was observed as that found by Siebenmann for the human, Asai for the dog and the rat, and Shambaugh for the pig, the sheep, and the calf. A single artery supplies the entire labyrinth, entering through the meatus acusticus internus. The first branch is a vessel which follows the ramus utriculus ampullaris and supplies the utricle and the anterior crurae of the superior and the horizontal canals. The opposite crurae of these two canals, the crus commune and the posterior canal are all supplied from a second branch. The first vessel is usually termed the anterior vestibular artery and the second the posterior vestibular artery. The relation of the latter to the vessel supplying the basal coil of the cochlea differs in different animals. Nabeya found in the guinea-pig that two parallel arteries supplied each crus of the semicircular canals. This relation has not been observed in other animals studied. Special interest lies in his findings regarding the venous system. Siebenmann had found three veins draining the human labyrinth. One vein left the labyrinth along the aquaeductus cochleae, a second along the aquaeductus vestibuli, and the third along the meatus acusticus internus. Shambaugh found in the pig all the veins of the labyrinth united into one vessel which left along the aquaeductus cochleae. This same condition was found in the sheep, while in the calf as an anatomical variation a small vein was occasionally found leaving along the aquaeductus vestibuli. In none of the animals

¹The original paper was so extended and had so many colored illustrations that it was decided by Professor Bensley not to attempt publication in American journals. Doctor Nabeya will publish the main paper in Japan, but since the text, in the Japanese language, will not be available to most of those interested in the results, the present abstract is printed here.

studied did he find a vein in the meatus acusticus internus which drained vessels from the labyrinth. Later Asai, working in the laboratory of Siebenmann, seemed to corroborate in the dog and the rat the veins described by Siebenmann for the human. Nabeya in his studies made a special effort to determine this question of the veins' leaving the labyrinth. His results are in accord with the observation of Shambaugh, that in both the guinea-pig and the cat all the veins draining the labyrinth are collected into a single trunk which leaves along the aquaeductus cochleae. In the rabbit, in addition to this vein, he found a vein draining the vestibule and the semicircular canals which leaves along the aquaeductus vestibuli. Only in the cat was he able to make out a vein in the meatus internus, which, however, he does not believe is a vessel belonging to the internal ear. The special method employed in his research left no possible chance for the destruction of these vessels, as Siebenmann and Asai have suspected in their criticism of the work of Shambaugh.

As a rule, there are two veins which drain the vestibule and the semicircular canals, the anterior vestibular vein draining the region supplied by the anterior vestibular artery and the posterior vestibular vein draining the region supplied by the posterior vestibular artery. Shambaugh had found in the sheep that the anterior vestibular vein was wanting and that all the veins from the vestibule and semicircular canals were collected by one trunk, the posterior vestibular vein. Nabeya found in the cat a similar arrangement by which a single vein drains the utricle and all of the semi-circular canals.

No blood vessels, as a rule, are found either in Reissner's membrane or connecting the vessels of the lamina spiralis with those of the spiral ligament, the zona pectinata of the membrana basilaris remaining free from vessels. Shambaugh had found in the pig that the membrane of Reissner was without blood vessels, but in the zona pectinata he found an occasional vessel connecting the spiral vessel under the tunnel of Corti with the vessels in the spiral ligament. In the sheep and the calf he found vessels in both the membrane of Reissner and in the zona pectinati. Nabeya found in the guinea-pig no vessels in Reissner's membrane or the zona pectinata. In the rabbit, however, he found vessels in both of these structures.

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THE ENDOTHELIAL PROBLEM¹

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FOREWORD

I think we now all recognize the general principle that the endothelium of blood-vessels may arise in loco in the body-axis of the embryo, a view which is opposed to the well-known angioblast or ingrowth theory of His. My speech this evening will be merely a survey of the direction taken by a long line of investigations, made chiefly by American anatomists, which has finally led up to the establishment and recognition of this fact. No further claim is made for the principle of a local origin of lymphatic endothelium from mesenchyme, beyond what has previously been stated in print. It will be necessary, however, to refer to certain investigations on the lymphatic system, as it is largely through them that we have been guided in arriving at the facts.

This seems an auspicious occasion to review this subject in general, not only as a tribute to the work of a large group of American anatomists, but also for the reason that such a review may serve as a guide to the younger generation of anatomists who may care to continue this work.

Since endothelium is the essential tissue of the vascular system, the question has naturally arisen as to the manner in which this endothelium makes its appearance in the course of ontogeny; also, where and when it arises in the embryo and how the endothelial-lined channels of the vascular system are established. Two opposing theories have been advanced by European anatomists in answer to these questions: A. The angioblast theory of His; B. The local-origin theory.

¹ President's address; read before the American Association of Anatomists, at their annual dinner, held in Philadelphia, on March 25, 1921.

A. THE ANGIOBLAST THEORY

It has been observed in meroblastic ova, like those of the domestic fowl, that blood-vessels and blood-cells seem to make their first appearance on the yolk-sac and then appear at a somewhat later stage of development in the body-axis of the embryo. The early appearance of the blood-vessels on the yolk-sac led His to infer that certain cells on the yolk-sac, which have been described by some as derivatives of entoderm and by others as derivatives of mesoderm, undergo an early differentiation to form a specialized tissue from which the endothelium of the yolk-sac vessels and the blood-cells are exclusively derived.

This supposedly precociously developed vascular tissue formed on the yolk-sac has been termed the *angioblast* by His, who regarded it as forming a local unit vascular anlage from which, in addition to the endothelium of the blood-vessels on the yolk-sac, that which appears within the body of the embryo is also directly derived. According to the angioblast theory, the yolk-sac angioblast grows into the embryonic axis from the yolk-sac in a continuous and uninterrupted manner, thereby supplying to the embryo all of the material which subsequently gives rise to the endothelium of the entire intraembryonic vascular system. This theory that the yolk-sac angioblast forms the unit vascular anlage of the entire vascular system precludes the possibility that the intraembryonic endothelium arises from any tissue in the embryonic axis other than the invading angioblast. The gradual and progressive manner in which the angioblast is supposed to grow into the embryonic axis also necessarily implies that no discontinuity between the angioblast on the yolk-sac and any portion of that which has invaded the embryonic axis can possibly exist at any time or place. The angioblast is therefore regarded as being a highly specialized tissue, early differentiated during embryonic development, and at first confined to a localized area, the yolk-sac. That all intraembryonic endothelium is derived from this local unit vascular anlage by a process of continuous growth, and that this fact marks the specificity of the angioblast and its derivatives, is one of the primary and fundamental axioms of the

angioblast theory. Another essential feature of the angioblast theory is that all intraembryonic endothelium invariably arises from some preexisting endothelium. If it did not arise in this manner, it would have a local origin from some tissue other than endothelium.

The implied specificity of yolk-sac angioblast, the origin from it of intraembryonic endothelium by a process of continuous growth, and the necessity thereby implied that all intraembryonic endothelium arises from some preexisting endothelium, constitute the logical claims of the adherents of the angioblast theory.

B. THE LOCAL-ORIGIN THEORY²

The view opposed to the angioblast theory is that of local origin. According to this view, mesenchyme may, in practically any region of the body, transform into vascular tissue. The cells which bound an intraembryonic blood-vessel are not in direct lineage with those which line the early vessels on the yolk-sac; they have not come into being as an ingrowth from the early yolk-sac vessels or angioblast; they have not necessarily come from preexisting endothelial cells, though some of them may have had such an origin, inasmuch as local origin does not preclude the possibility of growth during or following the process of local vascular formation. Addition to endothelium may take place, 1) by proliferation of endothelial cells already formed; 2) by addition of single mesenchyme cells; 3) by addition of solid cell aggregates; 4) by addition of already formed endothelial cavities, the lining cells of which have differentiated locally, in and from the mesenchyme, and, 5) by the active migration and alignment of single mesenchyme cells to form vascular cavities. The local-origin theory holds that blood-cells are not necessarily descended from a primitive yolk-sac angioblast, but that mesenchyme within the embryonic body is capable of giving rise to blood-cells. Advocates of the local-

² With only slight modifications, this account of the local-origin theory has been taken bodily from Reagan's paper, in volume 21 of *The American Journal of Anatomy*.

origin theory do not believe that the vascular anlagen are necessarily differentiated at a very early stage of development, as claimed by His, or collectively and at one time, as stated by Minot; advocates of the mesenchymal theory recognize that there are certain regions in which a precocious production of vascular tissues takes place, but they claim that such regions are not the only regions in which such tissues are formed. Advocates of local-origin theory recognize various intraembryonic regions in which there is a first-hand production of vascular tissues, even relatively late in ontogeny, quite independent of such processes in the yolk-sac. The angioblast theory regards endothelium as a tissue of high specialization quite foreign in nature to mesenchyme and quite removed from it genetically. The local-origin theory claims that mesenchyme can transform into endothelium and that endothelium can transform into mesenchyme.

The theory of local mesenchymal origin of endothelium dates back to the work of Reichert ('62) and Goette ('74). Within more recent times, Rückert and Mollier ('06) and their students have chiefly constituted the European School which maintains that endothelium develops *in situ*. Von Felix ('97), Maximow ('09), Bonnet ('12), and other European anatomists also have supported this view. In this country, Huntington and McClure, with their associates and students, have, until quite recently stood practically alone as sponsors of the local-origin theory.

Differences of opinion among European anatomists regarding these two views have been concerned primarily with the development of blood-vascular channels. The apparent reason for this is that until recently no consistent and uniform plan of development for the lymphatic system had been observed; therefore it was not possible to consider its development in the light of one or the other view. An examination of any one of the current text-books of embryology published prior to 1902 clearly shows how little was really known regarding the development of the lymphatic system up to that time. The general consensus of the opinions expressed, however, seemed to favor the local-origin view.

So far as I am aware, Florence Sabin was the first to emphasize a theory of development for the lymphatic system which could be consistently interpreted in the light of one of these two opposing views. In 1902 Sabin published her first paper on the development of the lymphatic system, in which its development was interpreted in the light of the angioblast theory, or the theory of continuous growth, which is opposed to the local-origin view. The significance of this paper lies in the circumstance that it presents in concrete form a theory of lymphatic development which, if proved to be correct, would seemingly confirm the angioblast theory of His, and would thereby establish the theory of a uniform plan of development common alike to all intraembryonic blood-vessels and lymphatics. The appearance of this paper may be said to have served as a stimulus to other investigators for the publication of a series of investigations extending over a period of fifteen years which, at first, were primarily concerned with the development of the lymphatic system, but which later inevitably included the development of the blood-vascular system as well.

By injecting India ink at definite points into the subcutaneous tissue of a consecutive series of pig embryos, Sabin attempted to show that all lymph vessels bud off from the veins at four primary centers and then invade the skin, as well as the deeper-lying regions, by a process of centrifugal growth. She states (p. 387):

It has now been shown that the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds and later from two similar buds in the inguinal region ducts grow toward the skin and widen out to form four sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line following the aorta to make a thoracic duct from which the lymphatics grow to the various organs. Thus the ducts of the lymphatic system gradually invade the body, but there are certain tissues which they never reach in the adult, for example, the cornea and cartilage.

It is evident that this theory of a continuous centrifugal growth of lymphatic endothelium from the endothelium of the veins is merely an extension and application to the lymphatic system of the angioblast or ingrowth theory of His. Viewed in the light of the angioblast theory, the endothelium of the lymphatics, like that of the blood-vessels, would possess a marked specificity. It would never arise discontinuously in situ from mesenchyme, but invariably in continuity with some preexisting endothelium whose origin could also be traced back directly and continuously to a unit vascular anlage known as the angioblast.

In 1906 Huntington and McClure read before the Association of American Anatomists a paper in which they claimed that the main lymphatic vessels of the cat do not appear to develop from the endothelium of the veins by a process of centrifugal growth, as maintained by Sabin, but develop in situ from mesenchyme. They also showed that certain of the main lymph channels of the cat, such as the thoracic duct, assume in the adult, a position, occupied in the embryo by functional venous channels which later undergo degeneration. This paper was published in volume 1 of the *Anatomical Record*, which appeared in 1907.

Following Sabin's first paper in 1902, nearly one hundred others have appeared in substantiation of one view or the other. Since 1906, the investigations concerning the histogenesis of endothelium, with the exception of an important paper by Hahn in 1909 and a very few others, have all been made by American anatomists. The following methods have been employed in the investigation of the problem:

1. The injection method;
2. Study of sections and reconstruction of uninjected embryos;
3. Study of sections and reconstruction of injected embryos;
4. Study of the living embryo;
5. Experimental method:
 - a. Isolation of parts of the embryo which are allowed to develop independently;
 - b. Chemical treatment of the embryo;

c. Study of the reaction of endothelium toward colloidal acid dyes in the living embryo (so-called method of vital staining).

While all of the papers published in connection with this problem are important in the sense that they have somewhat influenced its solution, still there are certain ones among them that have had a peculiar importance in directing the course of the investigation. It is to this latter group of papers that we shall for the most part give our attention.

In 1905, slightly previous to the first publication by Huntington and McClure, a paper by F. T. Lewis appeared on the development of the lymphatics in the rabbit. Lewis described the main lymphatics as being developed through the confluence of independent endothelial-lined sacs which he thought had become detached from the veins. While this paper appeared to coincide with the view that the lymphatics were derived from the endothelium of the veins, it also emphasized the circumstance that the main lymph channels did not grow out continuously and centrifugally from the endothelium of the veins, but were formed by the confluence of a number of independent anlagen.

This paper by Lewis influenced greatly the subsequent work of certain investigators. The angioblast theory and Sabin's conception of the development of the lymphatics demanded that these independent lymph sacs of Lewis should still remain in continuity with the veins, regardless of the fact that such continuity could not be actually demonstrated. In a paper published in 1908, Sabin, while finally recognizing the existence of these endothelial-lined lymph sacs of Lewis, asserted that they appeared isolated only in the study of serial sections, and still firmly maintained that their continuity could be demonstrated by the method of injection.

In 1908 Huntington and McClure read a preliminary paper on the development of the jugular lymph sacs in the cat. This paper appeared in its completed form in 1910. They observed that the jugular lymph sacs of the cat did not bud off from the veins in the neck as two blind ducts, as maintained by Sabin, but were derived from a preceding plexus of vessels which, at a number of points, communicated with the veins of the neck.

This plexus of vessels, out of which each jugular lymph sac consolidated, was termed a venolymphatic plexus, on account of its duplex relation to the lymphatics and veins. The investigation led Huntington and McClure to conclude at that time that the jugular lymph sacs were of venous origin and that they formed reservoirs, comparable to the lymph hearts of the lower vertebrates, into which the independently formed systemic lymphatic vessels opened before draining into the veins. The acknowledgment that the jugular lymph sacs were of venous origin seemed to the adherents of the angioblast theory additional evidence that the local origin theory was incorrect.

In 1908 McClure published also a paper on the development of the thoracic duct in the cat, in which he accepted the view advanced by Lewis, that the thoracic duct was developed through the confluence of a multiple series of endothelial-lined vesicles which had become detached from the veins. This view he later retracted in 1910.

In 1909 E. R. Clark published the first of a series of papers on the growth of the lymphatics in the living tadpole's tail. He observed that the lymphatics grew by a process of sprouting or budding from preexisting lymphatic endothelium, and also clearly showed that in such cases contiguous mesenchyme cells were not involved in the process. Although Clark did not describe any instance in which the lymphatics were found to bud or sprout from the endothelium of the veins, he believed that all lymphatics of the body were formed by a process of sprouting, and inferred accordingly that they originally sprouted from the endothelium of the veins. Clark's papers were regarded by the adherents of the angioblast theory as furnishing the final proof in favor of this view.

1909 marks also the beginning of the revival of the study of the development of the intraembryonic blood-vascular system. By the aid of the injection method, Evans concluded that a united vascular system was always present in the embryo, so that blood-vessels form a single though irregularly branched endothelial tree whose branches are in no case added after an independent formation, but arise always by sprouting from the parent trunk.

This view, as expressed by Evans, coincided with the angio-blast theory of His.

In 1910, at the International Anatomical Congress in Brussels, papers were read by Huntington and McClure on the development of the thoracic duct and the mesenteric lymphatics in the cat. The former paper was published in 1911 by Huntington in the form of an extensive monograph. In the latter paper McClure formally retracted the view previously expressed in 1908 that the thoracic duct was derived from the endothelium of the veins. These two papers described in greater detail than had hitherto been given the local origin from mesenchyme of the thoracic duct and mesenteric lymphatics.

With the exception of the work of E. R. Clark, the evidence for a venous origin of the lymphatics had up to this time been based almost exclusively upon the results obtained by the use of the injection method. This was the case with the American investigators, as well as with most members of the Polish School, led by Hoyer. Huntington and McClure had frequently called attention to the self-evident fact that the method of injection, even if successful, would only demonstrate the channels or spaces actually continuous with each other at the time the injection was made, and would completely fail to reveal vascular spaces, as yet independent of those injected, even though a connection between them might subsequently be formed.

This inadequacy of the injection method led to a more detailed study of serial sections of injected embryos in order to determine whether independent anlagen of the lymphatic system were present beyond the injected field. Stromsten was the first to analyze the subject critically from this standpoint in a series of three papers, the first one of which appeared in 1910. He injected the lymphatic system in a consecutive series of turtle embryos, and asserted that independent anlagen of the lymphatics were invariably present beyond the injected field.

With the exception of a paper by Huntington on the development of the lymphatics in reptiles, no actual advance in any direction, not already mentioned, was made in 1911.

Evidence in favor of the venous origin of the lymphatics and of the angioblast theory in general had apparently reached the high-water mark in 1911. Articles by Evans and Minot on the blood-vascular system, and by Sabin, on the lymphatics, appeared in Keibel and Mall's *Embryology*, definitely declaring in favor of the specificity of endothelium and the angioblast view. Also, two papers of a critical nature regarding the methods thus far employed by different investigators were published by Sabin and E. R. Clark in 1911.

In 1912 an investigation appeared by Kampmeier, who demonstrated the presence of independent anlagen of the thoracic duct in an injected pig embryo, which had been loaned to him by Sabin with the express purpose of furnishing evidence that such anlagen did not exist. A critique of this paper was published by McClure in 1912. Also in 1912 Sabin arrived at the conclusion that the thoracic duct was formed in part by a down growth from the jugular lymph sac, and in part through a fusion of independent endothelial-lined sacs, which, like Lewis, she still regarded, however, as detached veins. With the exception of a general review of the subject which appeared in 1913 and the Harvey lecture on the growth of the lymphatic system published in 1915, this is the last investigation, so far as I am aware, which has been published by Sabin on the development of the lymphatic system.

In 1912, E. R. and E. L. Clark published a series of observations on the living growing lymphatics in the embryo of the domestic fowl, and concluded that the posterior lymph hearts were derived from the veins. A paper on the development of the jugular lymph sac in the domestic fowl appeared also in 1912 by A. M. Miller, in which he claimed that the jugular lymph sac developed in situ from mesenchyme.

In 1912, Bremer also attempted to strengthen the angioblast theory by showing that the discontinuity described for the developing intraembryonic blood-vascular system did not really exist, as these apparently discontinuous elements were connected with one another by solid angioblast cellular cords.

Another paper of importance appeared in 1912 by Whipple and McWhorter, who were the first to follow in detail the development of the blood-vessels on the yolk-sac in the living embryo of the chick. They were able to demonstrate and to record photographically that the blood-vessels in the area pellucida were formed through the concrescence of separate and locally formed anlagen, and that the growth of endothelium was not continuous between the yolk-sac and the body-axis of the chick.

Between 1913 and 1915, McClure published a series of papers on the development of the lymphatics in fishes. In a consecutive series of injected trout embryos, he found that the main lymphatic channels were formed through a confluence of separate and independent endothelial-lined vascular spaces, or lymph vesicles, which could not be injected from the veins. Two of these independent anlagen, the subocular lymph sacs, were relatively huge structures which could be observed in the living embryo. By injecting India ink into these sacs in the living embryo it was observed in early stages that ink did not pass out of the sacs into the other portions of the lymph system or into the veins. A study of the development of these subocular lymph sacs showed that they develop in situ from mesenchyme; also that they only temporarily retain their independence as disconnected anlagen of the lymphatic system, and secondarily establish a connection with other independent lymph vesicles to form a continuous system of vessels, through which the lymph can then drain into the veins.

The chief significance of this investigation lies in the circumstance that one is actually able to demonstrate in the living trout embryo the presence of independent and discontinuous anlagen of the lymphatic system.

In 1913, Miller published a paper on the development of the thoracic duct in the domestic fowl. He observed that the periaortic mesenchyme of the chick is the site of a most active and abundant intraembryonic haemopoiesis in situ. He showed that masses of developing blood-cells differentiate axial strands around the aorta directly from the indifferent periaortic mesenchyme. Subsequently, the anlagen of the thoracic ducts appear

in this periaortic area as isolated intracellular clefts and spaces. These spaces become confluent, receive the blood-cells developed in the mesenchymal blood-islands, and convey them through the channels of the thoracic duct to the jugular lymph sacs, and through them into the circulating venous blood-stream. After this evacuation of their early blood contents, the axial lymphatic channels are retained as the permanent thoracic ducts. Lymph vessels which convey developing blood-cells to the circulating blood-stream, Miller designated as haemophoric lymph vessels.

The presence of haemophoric lymph vessels in the embryo of the domestic fowl led Huntington to make a further detailed study of the development of the jugular lymph sacs in the cat, in the early embryonic stages of which blood-cells had previously been observed by McClure and Huntington in certain disconnected anlagen.

In a paper published in 1914 Huntington showed, by means of an improved technique, in stages earlier than those hitherto studied by him and McClure, that the anlagen of the jugular lymph sacs, like those of the thoracic ducts in the chick, first appear in the midst of an extensive haemopoietic field as isolated intercellular clefts or spaces. When these spaces become confluent to form the jugular sacs, the walls of the latter enclose great masses of blood-cells. The jugular sacs then temporarily connect with the veins at a number of points at which the blood-cells are evacuated into the blood-stream. After the evacuation of this blood content, each jugular sac temporarily becomes detached from the veins, but later establishes a connection with them at two typical points.

Huntington also showed that in the embryo of the cat the primitive ulnar lymphatic functions temporarily as a haemophoric vessel, which later undergoes complete atrophy. This investigation by Huntington emphasized two important points: first, that the presence of blood-cells in the lymphatics does not necessarily signify a venous origin of the latter, and, second, that the jugular lymph sacs of the cat, like the main systemic lymphatics, are developed in situ from mesenchyme.

Huntington's paper was followed by a preliminary account by West on the development of the posterior lymph heart in the domestic fowl. The final paper was published in 1915. West asserted that the posterior lymph heart of the domestic fowl develops in situ from mesenchyme and, like the thoracic ducts of the embryo, possesses a haemophoric function during the early stages of development. The results of this investigation were not in accord with those previously obtained in 1912 by the Clarks who maintained that the lymph heart was derived from the veins.

Two papers appeared in 1914 on the development of the intraembryonic haemal endothelium by Schulte and Bremer, respectively. Schulte's publication appeared to be even more convincing than any morphological investigation hitherto published which had dealt with the genesis of intraembryonic haemal endothelium. He proved in a most decisive manner that the umbilical vein of the domestic cat develops in situ from mesenchyme, and is not derived from yolk-sac angioblast. In his 1914 paper Bremer modified his earlier conception of the angioblast theory. He claimed that the angioblast might also arise in the embryo independently of that on the yolk-sac, its only prerequisite being that it shall grow continuously from one or many sources of origin through the embryonic tissue as solid cords of cells. This later view of Bremer's could be interpreted as a recognition of the principle involved in the theory of the local origin of endothelium, as this theory does not deny the possibility of the growth of endothelium after it has once been locally formed.

1914 marks the termination of a period in which the investigations were almost exclusively morphological in character, and the beginning of one in which certain of these investigations have been confirmed from the standpoint of experiment.

It will be observed that from 1914 up to the present writing but two investigations have appeared in print in which a claim has been made that intraembryonic haemal endothelium is derived from yolk-sac angioblast (Wislocki, '16) or that lymphatic endothelium is a derivative of the endothelium of the veins (Wislocki, '16; E. R. and E. L. Clark, '20).

From 1914 on, the question of the specificity of endothelium in particular, and that of the continuous growth theory of endothelium in general, has seemed to lack its former support.

Two important experimental papers appeared in 1914 by W. C. Clarke and by Miller and McWhorter, respectively. Clarke's paper was concerned especially with the question of the specificity of endothelium. He showed that vascular endothelium, wherever encountered, haemal or lymphatic, may be an instance of the environmental adaptation of an originally isodiametric mesenchymal cell subjected to mechanical influence. He observed that unilateral pressure, e.g., the accumulation of fluid under tension in intercellular spaces, will produce flattened modified mesenchymal cells which resemble endothelium. If the pressure is released, the former endothelial cell will promptly revert to the type of the indifferent mesenchymal cell, of which it is merely an adaptive form, modified in accordance with definite hydrostatic and other purely mechanical factors. Consequently, he claimed that the modified mesenchymal endothelial cell loses all pretensions to specificity in the sense implied by the angioblast theory.

Miller and McWhorter cut off the lateral half of the entire area opaca of the blastoderm in developing chick embryos at a time when no blood-vessels or so-called angioblast had appeared in the area pullucida or embryonic body, and the blastoderm was then allowed to proceed in development. After a period of incubation lasting from twenty-four to forty-eight hours, the embryos were sectioned and graphic reconstructions made of the vascular system in the embryonic body. Blood-vessels were invariably found on the operated or injured side as well as upon the uninjured side of the body. Essentially similar experiments had previously been made by Hahn in 1909 with the same results. Hahn's paper had not been brought to the attention of Miller and McWhorter, however, until after their own experiments had been completed. The results of both sets of experiments seemed to leave little doubt as to the validity of the view that the intraembryonic blood-vessels are not derived from yolk-sac angioblast, but arise in situ within the body-axis of the embryo.

Following the publication of Miller and McWhorter's paper, the objections urged against it from certain quarters included the following: the incisions may not have been made sufficiently close to the embryonic axis or may not have been made sufficiently early and endothelium may have grown in from the opposite side or from the ends. In order to satisfy these objections, Reagan was able in 1915 to isolate completely a portion of the chick's embryonic body from all the outlying blastoderm at a time before the embryonic tissue is vascularized. After a period of incubation he found that endothelial-walled vessels or spaces were invariably present in such meroplasts.

In 1915, Stockard observed in chemically treated living teleost embryos, in which development of the vascular system had been arrested, that vascular endothelium arises in loco in many parts of the embryonic body. Stockard also observed in the living embryo of *Fundulus* the active migration of mesenchyme cells from the body-axis on to the yolk-sac, where they entered directly into the formation of the endothelium of blood-vascular channels.

Similar results were subsequently obtained also by Reagan in 1915-17 in hybrid teleost embryos and in normal and chemically treated living embryos of *Fundulus*.

In 1915 McClure published a critical review of the investigations on the histogenesis of the vascular system which had appeared up to that time.

In 1916, following the method employed by Stockard and Reagan, McClure observed in chemically treated living embryos of a teleost (*Erymizon*) that development of the lymphatic and blood-vascular channels could be arrested at a stage in which both were represented by completely independent and discontinuous anlagen. This investigation by McClure on teleost embryos showed that the local origin of lymphatic endothelium from mesenchyme, like that of the blood-vessels, could be determined by experiment. While this investigation has been completed, it has not as yet been published in full.

On the basis of the discovery that trypan blue is an elective stain for amphibian embryonic lymphatic endothelium, Wislocki

claimed, in 1916, that the different theories of lymphatic development could be subjected to a crucial test. Wislocki held that lymphatic endothelium was derived from the veins.

The 'crucial' tests regarding the theories of lymphatic development referred to above by Wislocki were made by McClure and published in 1918 in *The American Anatomical Memoirs of The Wistar Institute of Anatomy*. As stated by McClure: Evidence from the standpoint of vital staining by colloidal acid dyes that the lymphatics grow out from the veins, would at least call for a demonstration of an early stage of growth, at which a vitally stained lymphatic is in the process of growing out from a vein. I may state that not even the faintest approach to such a demonstration can possibly be realized in the embryo of either the frog or the toad. The reason for this is plain. The lymphatic system of the frog and the toad does not become vitally stained, so to speak, by colloidal acid dyes, until an extensive system of continuous lymphatic vessels have been formed, which convey lymph from practically all regions of the body to the anterior lymph hearts and thence to the veins. It is also significant to note that the endothelium of the anterior lymph hearts in the frog and toad, from which, by some investigators, the lymphatics are supposed to grow, is not vitally stained by colloidal acid dyes. As a matter of fact, the earliest stage of development at which the lymphatics react toward these dyes in the frog and toad is one not very far removed from their permanent larval form. Such an advanced stage would not ordinarily be regarded as a favorable one from which to infer in what manner the lymphatics have been formed.

The method of vital staining by colloidal acid dyes, like the injection method, as ordinarily used, does not therefore demonstrate the existence of lymphatics in the embryonic body until their development is far advanced and until continuous channels are formed. This method, like the injection method, is incapable of demonstrating the early independent anlagen of the lymphatics, like those of the head sinuses in the frog and toad, which at one time are entirely independent of each other and of the veins. The 'crucial' test referred to by Wislocki therefore completely

fails, as a vital staining of the lymphatic tissues by his method is not possible at a time when the earliest anlagen of the lymphatic system first arise.

It was also shown that lymphatic endothelium is not the only tissue which reacts toward colloidal acid dyes. The reaction is therefore not specific in the sense implied, but rather an attempt on the part of the lymphatics and certain other tissues to rid the blood-stream of the dye.

In 1917, Sabin definitely declared in favor of the local origin of intraembryonic haemal endothelium—a view which she opposed in 1913. Her present position regarding the development of the lymphatics, so far as one is able to judge, is similar to that stated by her in 1913. In 1920 she published also an elaborate monograph which appeared in the Mall Memorial Volume, in which she described in considerable detail the local origin of intraembryonic blood-vessels and red corpuscles as observed in the living embryo of the chick.

If we analyze the results of the above-mentioned investigations, it is evident that the morphological evidence favoring the general principle of a local origin of intraembryonic endothelium from mesenchyme has been completely confirmed by experiment, and that the angioblast theory, in the sense maintained by His, therefore no longer holds.

While differences of opinion may still exist, as regards details of the process, both for the lymphatic and blood-vascular systems, it is plain from this brief sketch, that the general principle of the local genesis of intraembryonic endothelium from mesenchyme, a theory so recently and so vigorously opposed by a large group of American anatomists, may now be regarded as an established fact.

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Resumen por el autor, C. V. Cowdry.

La tendencia conservadora en la nomenclatura citológica.

El autor considera con cierta aprehensión la multiplicidad creciente de los términos citológicos. Un análisis demuestra que algunos de ellos han sido introducidos para indicar ciertos atributos morfológicos; otros para designar ideas relativas a la constitución química; otros, de nuevo, para recordar los descubridores (introduciendo de este modo todo el problema de la prioridad) mientras que los más confusos han sido ideados para proclamar alguna interpretación funcional más o menos teórica. La confusión resultante es perjudicial para el pensar claro y retarda definitivamente el progreso de la citología, pero ha de empeorar aún más hasta que los investigadores se hagan cargo suficientemente de la situación, considerando la introducción de nuevos términos sin buenas razones, como una especie de ofensa. Un cierto grado de tendencia conservadora es necesario. El autor indica que sería útil que alguna asociación nacional determinase los principios que han de inspirar la nomenclatura citológica para uso de los investigadores.

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CONSERVATISM IN CYTOLOGICAL NOMENCLATURE

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Repeated and ill-advised invention of new terms to designate cytoplasmic structures has resulted in considerable confusion.

A pathologist, for instance, who wants to review the literature on mitochondria is apt to be discouraged when told that, in order to be on the safe side, he must look for papers under at least a dozen different headings: bioblasts, chondriosomes, plastochondria, etc.

It is not without reason that biochemists and physiologists, who are able to help so much, are content to remain ignorant of advances in cytology. They are confronted by a mass of almost meaningless words which cannot be defined with anything approaching the accuracy to which they are accustomed. All too frequently they meet hypotheses which are absurd in the light of recent advances in physics and chemistry. A barrier is built up where the closest coöperation is needed. New and delicate methods are being devised for the measurement of temperature, electrical potential, ionization and other phenomena, which are of the utmost value to cytologists, but which their limited knowledge of physics and chemistry prevents them from using. Not infrequently the inventors themselves are unaware of the nature of the problems for which they are unconsciously supplying the means of solution.

The cytoplasmic material commonly called mitochondria (Benda, '98, p. 397) presents certain general properties, though the fact that it varies slightly in different cells suggests that it is in reality a mixture of chemical substances:

1. It may be easily seen to occur in living cells in the form of granules, rods, and filaments, and occasionally of networks, which vary in shape and in number in different conditions.

2. It gives a characteristic color reaction in living cells when janus green B (diethylsafraninazodimethylanilin) is applied in a solution as weak as 1:500,000. It first assumes a bluish-green color, then turns pink (diethylsafranin), and finally bleaches to the leucobase. The delicacy of the reaction is shown by the fact that janus green (Grübler) and janus green C will not stain mitochondria specifically, though they differ only in the substitution of an H_2 or $(CH_3)_2$ group in place of the (C_2H_5) .

3. Its solubilities are characteristic, and in fixed tissues it stains more or less specifically by the methods of Altmann, Benda, Bensley, and others.

The term 'mitochondria' is well known and has been widely used for over twenty years. It indicates variability in morphology, from threads to granules, and carries no theoretical or functional interpretation whatsoever (Cowdry, '18, p. 47).

Mitochondria did not altogether escape the older authors, who, with their relatively crude methods, observed and described them, often in confusion with materials of quite different nature, under many terms of which the following may be mentioned:

Archoplasma-schleifen (Hermann)	Neurosomen (Held)
Basal filaments (Solger)	Nucleus of Balbiani (Van der Stricht)
Centrophormien (Ballowitz)	Plastidulen (Maggi)
Chromidia ¹ (Hertwig)	Plasmosomen and Plasmomiten (Arnold)
Cytomicrosomes (Heidenhain)	Protoplasma supérieur (Prenant)
Ergastoplasme (Garnier)	Pseudochromosomen (Heidenhain and Van der Stricht)
Fila (Flemming)	Q and J granules (Holmgren)
Fuchsinophile granules (Galeotti and others)	Sarcosomen Retzius
Granula (Metzner)	Sphären (Rhode)
Granules (of Schridde)	Sarcosomen (Retzius)
Interstitial-Körner (Henle and Koelliker)	Sphéroplastes of Protozoa (Fauré-Fremiet)
Leucoplastids (Schimper)	Vermicules (Laguesse)
Microsomes (Van Beneden)	Vegetative filaments (Altmann)
Mitom (Flemming)	Vitellogene Schicht (Van der Stricht)
Nebenkern (Butschli)	
Nematoplasten (Zimmermann)	

¹ In the days of confusion with 'chromidia' it is highly probable that mitochondria were described under this heading, though, in reality, they are wholly different.

Since their recognition as a definite class of cell granulation the literature has been flooded with new terms introduced by the authors with the best intentions and, in each case, in the firm belief that they would finally clarify the situation:²

Benda ('98, p. 397)	Chondriomitom	A feltwork of mitochondria
Benda ('99, p. 382)	Chondriomiten	Thread-like mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriorhäden	Shaft-like mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriosomen	A generic term to include all forms of mitochondria
Benda (Van der Stricht, '04, p. 105)	Chondriosphären	Spherical mitochondria
Renaut and Dubreuil ('06, p. 230)	Perinème	Mitochondria as they occur in certain connective tissue cells
Renaut and Dubreuil ('06, p. 230)	Pericaryonème	Mitochondria as they occur in certain connective tissue cells
Koltzoff ('06, p. 468)	Mitogels	Large mitochondrial masses formed by confluence
Koltzoff ('06, p. 468)	Mitosols	Mitochondrial droplets
Koltzoff ('06, p. 468)	Mitochondrosols	Mitochondrial droplets
Duesberg ('07, p. 284)	Mitochondrial apparatus	A general term, same as chondriom. (Duesberg, '08, p. 261)
Meves ('07, p. 401)	Chondriokonten	Rod-like mitochondria
Meves ('07, p. 403)	Chondriom	Cytoplasmic content of mitochondria
Giglio-Tos and Granata ('08, p. 14)	Chondriotaxie	The arrangement of granular mitochondria in threads
Giglio-Tos and Granata ('08, p. 1)	Chondrioderese ³	Division of mitochondria
Regaud ('09, p. 921)	Eelectosomes	To indicate supposed property of picking up substances from the surrounding medium and elaborating them
Meves ('10, p. 150)	Plastosomen	Generic term to include all forms of mitochondria
Meves ('10, p. 150)	Plastochondrien	Granular mitochondria supposed to play a definite part in histogenesis

² Rosenstadt's ('18, p. 193) 'tetrasomen' terminology is difficult to correlate.

³ Comes (p. 423) claims priority over Giglio-Tos in the invention of this term. Since, however, he confuses the mitochondria with the reticular material, it is not clear under which heading it should be given.

Meves ('10, p. 150)	Plastochondriomiten	Rows of granular mitochondria
Meves ('10, p. 150)	Plastoconten	Long rod-like mitochondria
Laguesse ('11, p. 276)	Ergastidions	To indicate supposed relation to ergastoplasm
Romeis ('12, p. 139)	Chondriolysis	Solution of mitochondria
Comes ('13, p. 423)	Condrio-cinesi	Changes in mitochondria during cell division
Comes ('13, p. 423)	Condrio-dieresi	Changes in mitochondria during cell division
Comes ('13, p. 423)	Idio-condrioma	Mitochondria supposed to play a definite part as carriers of heredity
Comes ('13, p. 423)	Trofo-condrioma	Mitochondria supposed to be concerned in nutrition
Asvadourova ('13, p. 293)	Chromochondries	To indicate supposed part in pigment formation
Wildman ('13, p. 428)	Karyochondria	To indicate supposed nuclear origin
Ciaccio ('13, p. 725)	Plastolysis	Solution of mitochondria
Ciaccio ('13, p. 725)	Plastorhexis	Breaking up of mitochondria into granules and vesicles
Ciaccio ('13, p. 725)	Präplastorhexis	Changes preliminary to plastorhexis
Champy ('13, p. 157)	Chondrioplastes	To indicate supposed part in formation of plastes
Arndt ('14, p. 55)	Caryosomochondria	To indicate supposed origin from the caryosome
Jordan and Ferguson ('16, p. 94)	Myochondria	To indicate supposed mitochondrial origin of myofibrils
Gatenby ('18, p. 223)	Micromitochondria Macromitochondria	Small mitochondria which are less dense than the larger ones and behave differently in the spermatogenesis of certain invertebrates
Gatenby ('18, p. 210)	Macromitosome	Used synonymously with the mitochondrial coil (nebenkern or paranucleus) of Lepidoptera
Meves ('18, p. 273)	Chondriomer	Suggests termination 'er' for purely morphological nomenclature
Guilliermond ('19, p. 239)	Mitochondries elaboratrices	To indicate mitochondria supposed to differentiate into chondrioplastes in the animal cell and into plastides in the vegetable cell

Guilliermond ('19, p. 239)	Mitochondries vegetatives	To indicate non-functional mitochondria which are thought to perpetuate the chondriome
Dangeard ('19)	Plastidome	(Quoting from Guilliermond '21, p. 73)

The reticular material cannot be so precisely defined, but we do know that under certain conditions it (or its mordant) has an affinity for silver; that after long immersion it blackens with osmic acid; and that, when fixed by appropriate methods (Bensley, '10, p. 191), it presents the form of a system of clear uncolored spaces. It may also be recognized by its morphology and by its position in the cell. Golgi ('98, p. 1) was the first to refer to its reticular shape. By substituting the general term 'material' in place of 'apparatus,' the idea of a definite system of canals is not sustained (Cowdry, '21, p. 8).

Like the mitochondria, it was occasionally seen by the older authors and described, together with other materials under many headings of which I have selected the following:

Archoplasm (Boveri)	Nebenkern (Butschli)
Boyaux incolores (Van Beneden)	Primitivröhrechen (Nansen)
Centrodeutoplasm (Erlanger)	Vasa serosa intracellularia (Adamkiewicz)
Cytozoon (Gaule)	Zentralkapsel (Heidenhain)
Mitosome (Platner)	

Stimulated by the discoveries of Golgi, Holmgren, and Kopsch, new terms are being devised wholesale:

Holmgren ('99, p. 139)	Saftkanälchen	Reticular material as clear canals (negative picture)
Nelis ('99, p. 102)	Spiremes	Reticular material in the form of clear, uncolored spaces
	Etât spiremateux du protoplasma	Reticular material in the form of clear, uncolored spaces
	Bandes incolores	Reticular material in the form of clear, uncolored spaces
Kopsch ('02, p. 934)	Binnennetz (Netzapparat)	Blackened reticular material
Holmgren ('03, p. 9)	Trophospongium	To indicate canals which penetrate the cell from the exterior. Evidently not the reticular material, which is purely cytoplasmic, though sometimes applied to it.

Cajal ('04, p. 25)	Aparato tubiliforme	Same as 'red endocellular de Golgi'
Fuchs ('04, p. 501)	Fadenknauel	Same as Saftkanälchen or reticular material in the form of clear, uncolored spaces
Sánchez ('07, p. 167)	Appareil réticulaire de Cajal-Fusan	Homologue of 'Appareil de Golgi-Holmgren'
Cajal ('08, p. 123)	Conduits de Golgi-Holmgren.	Reticular material as clear canals
Perroncito ('09) (Duesberg, '11, p. 884)	Dittocinesi	To indicate the process of breaking up of reticular material into fragments during mitosis
	Dittosomi	The individual fragments
Bensley ('10, p. 179)	Canalicular apparatus	Reticular material as clear canals
Maccabruni ('10, p. 447)	Retzius-Holmgren-schen Kanälchen	To indicate tubular nature and discovery by Retzius and Holmgren
Kuschakewitsch ('13, p. 310)	Sphärosomen Sphärotheca	Reticular material during a phase of spermatogenesis
Terni ('14, p. 68)	Formazioni periid- iozomici	Reticular material grouped about the centrosome
King (Gatenby, '16, p. 418)	Acroblasts	"Dictyosomes or individual units of the Golgi apparatus" (Gatenby and Woodger, '21, p. 279)
Stockard and Papanicolaou ('18, p. 39)	Idioectosome Idiophthartosome	Reticular material as it appears in certain stages of spermatogenesis
Gatenby ('18 a, p. 403)	Chondrioplasts	Same as 'Golgi rods' (i.e., rod-like masses of reticular material)
Penfield ('20, p. 303)	Retispersion	To indicate dispersion of the reticular material to the cell periphery
Penfield ('20, p. 303)	Retisolution	Solution of the reticular material
Saguchi ('20, p. 388)	Intracellular network	"The apparatus which can be made manifest by various methods, such as Kopsch's and Wiegler's osmium method, Sjovall's formalin-water-osmium method, Cajal's uranic nitrate-silver method, and Golgi's arsenic method"
Saguchi ('20 a, p. 15)	Urano-argento- phile apparatus	"Filamentous or granular corpuscles which can be made manifest by the Cajal uranic nitrate method"

The so-called 'chromidal substance' is another cytoplasmic constituent of wide distribution encountered by many investigators, who, failing to realize the substantial similarity of the fundamental vital processes in different tissues, have called it all kinds of names. Specific granulations, occurring only in certain cell types, give us much less trouble.

We cannot take refuge in priority, which is very difficult to establish, because modern cytology is resolving itself more and more into a gradual approximation to a logical interpretation of living materials observed for the first time years ago with the aid of Abbe's condenser and the newly introduced apochromatic lenses. Newly recognized protoplasmic constituents are, for the most part, specific in nature, like secretion antecedents, not general in distribution, like the mitochondria, and are only revealed by refinements in microchemical technique. It is but natural that investigators should introduce terms indicative of their theoretical conceptions, but the habit is a bad one, because our ideas are changing so rapidly. Altmann's choice of the term 'bioblasts,' to indicate his belief that small fragments of material, which we now know to be mitochondria, were independent micro-organisms, attracted so much criticism and ridicule that his valuable discoveries were soon forgotten and investigations along this line were set back many years. Terms of this kind indicative of the supposed functional activities of cellular structures are often invented one year only to be cast aside the next, but they remain in the literature to mystify visitors from other sciences.

I question also the wisdom of using special terms to indicate slight differences in morphology, which after all can only be detected with oil-immersion lenses. In the case of mitochondria, we are dealing with fragments of material of microscopic size. Changes in form occur from moment to moment and are merely the expression of interaction between the mitochondria and the surrounding medium; only rarely do they indicate noticeable differences in chemical constitution.

Realizing that the cell is a living unit, which is changing continually, our terms should, it appears to me, be general and comprehensive, not limited and specific. While the materials in

question continue to defy chemical analysis, the probability that they undergo progressive and important chemical changes, which are not revealed by our imperfect methods, should be entertained, and speculations as to their physiological rôle should be advanced with caution. Most of the structural differentiations recognized by cytologists are mixtures, not pure chemical substances, so that the problem is by no means a simple one.

Perhaps the greatest difficulties are met with during cytomorphosis. In spermatogenesis, for instance, the mitochondria appear to change into some kind of material fairly resistant to acetic acid and of different morphology and staining reaction. At what stage should we cease calling them mitochondria and speak only of the product, whatever it may be? The same difficulty is met with in the case of the reticular material. The introduction by Stockard and Papanicolaou ('18, p. 39) of the following terms, in connection with the development of the idiosome, is, in my opinion, of questionable value: *calyptosome*, *karyogranulomes*, *idiogranulotheca*, *idiophthartosome*, *idiosphaerosome*, *idiosphaerotheca*, *cytogranulomes*, *cryptosome*, *idioectosome*, *idioendosome*, *idiogranulomes*, *spermiocalyptrotheca*. I am inclined to agree with Duesberg's ('20, p. 8) usage in this connection. Since we are ignorant of the chemistry of the cytomorphic change, we have to come to some arbitrary decision in each instance, for every cell in the body as it grows old presents the same problem with respect to all its structural components.

So many and so varied are the structures which can be recognized in the nucleus and cytoplasm in normal as well as pathological conditions, the chemistry and physics of which we know next to nothing, that hundreds of terms are in every-day use, many of them synonymous. Specialization is carried so far that students of the cytoplasm become bewildered when first they interest themselves in nuclear structure, and investigators familiar with the nucleus have to go slowly in the terminology of leucocytic granulations and secretion antecedents, if, indeed, they ever consider them.

Our main problem is not an etymological one, but confusion in nomenclature reflects inaccuracy in methods of cytologic

analysis and in the coördination of our knowledge of cellular physiology. It arises from the fact that our information regarding the chemistry of protoplasm is largely indirect and deducive. With the discovery of new methods of direct analysis and the strengthening of the mechanistic point of view, that we must try to explain vital phenomena in terms of physics and chemistry, investigators will be less ready to advance theories without carefully considering the chemical changes which they hypothecate, or to make unqualified statements of the transformation of one cytoplasmic material into another without knowing the constitution of either and the probability or improbability of such a change. With more accurate knowledge will come conservatism; the useful terms will gradually be sorted out and the bad ones consigned to oblivion. But this rapprochement between cytology and the more exact sciences will be a slow process extending over many years. In the meantime are we to follow the command of the old Chinese philosopher, Lao Tzu: "Practise inaction; content yourself with doing nothing"?

Some will say that it is not a matter which warrants group action, that the individual must continue to be a law unto himself, and that the very haziness of our knowledge of cellular structure will make it impossible to arrive at any *modus operandi*. The Basel anatomical nomenclature of visible structural components, a much easier task, has on the whole been of the greatest help. In chemistry, certain conventions in terminology are recognized even in respect to compounds about which very little is known and concerning which there is much speculation.

Individual preference will of course continue to be followed, but I cannot help thinking that an attempt by some national organization, or group of individuals, not to dogmatize further on terms, but rather to determine guiding principles in cytologic nomenclature for the use of investigators would be desirable. To yield the best results this committee or group should consist of students interested in the structure of both the nucleus and the cytoplasm as well as of a botanist, a protozoologist, and a biochemist, because cytology, dealing as it does with the minute structure of living material, is the broadest of biological sciences.

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Resumen por el autor, Charles C. Macklin.

Nota preliminar sobre el cráneo de un feto humano de 43 mm. de longitud máxima.

El presente trabajo comprende una breve descripción del condrocráneo, esqueleto del arco branquial, vértebras cervicales y huesos de membrana del feto humano núm. 886 de la colección de la Carnegie Institution de Washington, basándose en veintiocho modelos y una reconstrucción de perfil. Las porciones cordal y precordal del tallo central forman un ángulo de 115° . Los centros de preosificación se mencionan. El cartílago supraoccipital esta osificándose excepto en los bordes, de los cuales parten los procesos ascendente y descendente. El autor da una explicación de la equivocación de Bolk al interpretar esta región. El surco occipitoparietal separa parcialmente la placa parietal de la escama occipital situada debajo. Los arcos neurales de las vértebras occipitales están bastante acusados, existiendo tubérculos yugulares marcados y procesos paracondiloides. El cartílago supracoclear falta. El proceso estiloides está unido con la cápsula por medio de cartílago. El proceso mastoideo es un pequeño nódulo libre. El orificio perilinfático está dividiéndose por los procesos intraperilinfáticos anterior y posterior, que se aproximan. Las alas hipoquiasmáticas son pequeñas. Las comisuras prequiasmáticas son precartilaginosas y los orificios del mismo nombre son relativamente grandes. La comisura alicoclear o línula en vías de desarrollo es completa, pero muy delgada. En la región etmoidal no se encontraron cartílagos paraseptales superiores. El proceso cupular es largo y precartilaginoso. En el meato medio se encuentra un proceso precartilaginoso delgado y largo. El autor ha modelado y descrito el sistema del conducto nasolacrimal del mismo modo que muchas de las demás estructuras con él relacionadas, tales como el notocordio, nervios principales y ganglios, etc. Se han representado todos los huesos de membrana con excepción del nasal.

Translation by José F. Nonidez
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PRELIMINARY NOTE ON THE SKULL OF A HUMAN FETUS OF 43 MM. GREATEST LENGTH

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Studies on the developing skull of man and the lower forms have recently gained an impetus through the appearance of several papers. Among the contributions from American laboratories may be mentioned those of Kernan ('16) and Lewis ('20) on the human skull, Terry ('17) on the cat, and Rice ('20) on the lizard. Although a great amount of work has already been done, yet the science of craniogenesis is only in its infancy. Of particular service has been the wax-plate method of reconstruction, and in recent years the refinement of this method represented in the plaster of Paris technique (Lewis, '15) has become a very valuable aid. It is of interest to pursue the study of the development of the human skull not only for its own sake, but even more on account of its relation to the larger field of the development of the head, and to the still larger domain of evolution, and I offer this preliminary note upon the developing skull of a human fetus already well advanced in the scale of differentiation in the hope that it may prove helpful in advancing our knowledge of cranial morphogenesis.

The Embryological Collection of the Carnegie Institution of Washington presents splendid opportunities for the investigation of almost any problem in human antenatal development, and I was fortunate in being able to avail myself of its privileges. I chose for study human fetus no. 886, of 43 mm. greatest length, because it represents a stage sufficiently near that of the 40-mm. fetus which I described some years ago (Macklin, '14) to provide interesting material for comparison, and yet sufficiently far from my earlier specimen to avoid duplication of the work of research. No. 886 was obtained in perfect condition and was exceptionally

well prepared. The sections were cut in the frontal plane at a thickness of 100 μ and the series is practically perfect. The models were done in plaster of Paris, and number twenty-eight. They are faithful reproductions of the original structures.

A model of the entire skull, cervical vertebrae, and cartilaginous branchial arch skeleton was first made at a magnification of ten diameters, and this was used for the grosser studies. The central stem and the right half of the occipital cartilage were also made at the same enlargement. For the examination of the minuter details, special models were made of selected parts, taken from the right side of the skull, and enlarged twenty diameters. In addition to the cartilage, the membrane bones of the right side were modeled, together with the closely related soft parts, as the nerves, vessels, mucous membranes, etc., to be referred to in the description. Profile reconstructions were also made, showing the texture of the frontal and parietal bones, and the relation of the skull to the brain and to the external form.

DESCRIPTION

The skull, in general form, is much like that of the 40-mm. human embryo from the collection of Professor McMurrich, of the University of Toronto, known as 'I^a Toronto,' which I formerly described (Macklin, '14), and which will be referred to in this paper as Ia. No. 886, however, is noticeably less developed than Ia, although the latter is shorter, and this apparent discrepancy is to be explained by the fact that the dimension of Ia was obtained by crown-rump measurement, while in the case of no. 886 it was greatest length.

Development is proceeding most rapidly in the anterior parts, notably in the ethmoidal region, judging from the character of the cartilage and from a comparison of earlier and later stages, as that of Lewis ('20), embryo no. 460, of 21 mm., and Ia.

Indications of future ossification centers

The cartilage is mostly mature, and it is practically a continuous mass. In ten regions (four paired and two unpaired) it is undergoing the change preliminary to ossification, although in no

case is there actual bone formation present. This change in the cartilage is most marked in the case of the center for the supraoccipital cartilage, which is single and involves the entire thickness of the plate, but not the entire width, there being a narrow uncalcified edge along the upper border and a short blunt point (the descending process) projecting downward into the foramen magnum. The paired centers for the exoccipitals are situated in and behind the jugular tubercles. These tubercles are distinct ridges which lie just lateral to the canals for the hypoglossal nerves and run back to the posterior condyloid notches on the border of the foramen magnum. At these notches the cartilaginous change involves the entire thickness of the plate.

In the basioccipital cartilage is a small area of modified cartilage which represents the single center of the basioccipital bone. In the temporal wings the cartilage is still less modified, but paired centers can be made out. The sphenotic centers are just beginning, and the cartilage here, at the outer ends of the alar processes, shows only a very small degree of change. There are paired centers near the lower ends of Meckel's cartilages, and here the perichondrium is ossified and represents part of the mandible.

The *central stem* is bent at the body of the sphenoid, the angle between the chordal and prechordal parts being 115° . It is thus narrower than in no. 460, where my measurement from Lewis's figures shows it to be 125° . The bodies of the cervical vertebrae make with the chordal part of the central stem an angle of 125° . This is probably a more variable angle. The corresponding angle in 460 was 110° .

The *foramen occipitale magnum* is relatively larger than that of Ia, and the tips of the occipital vertebral arches are farther apart, making the superior incisure much wider. These arch tips are the same distance apart as those of the atlas, but as we proceed down the spinal column the arch tips come closer and closer together. Those of the seventh cervical vertebra, however, are still some distance apart. Closure of the cervical spinal canal is evidently following the familiar course, in being completed earlier below than above.

It seems possible that the occipital vertebra may always present a spina bifida, the arch tips not coming into actual contact and fusing to complete the foramen magnum behind, as I formerly held. This is undoubtedly the case in certain dogs, notably of the short-nosed type. Dr. Adolph H. Schultz has shown me mature skulls of bulldogs and pugs, nos. 71, 381 and 382 of his collection, where the extremities of the occipital neural arches are separated by a distinct interval and where, accordingly, the superior occipital incisure has persisted. It is a conspicuous notch which projects dorsally as an extension of the main part of the foramen occipitale magnum.

The *occipital vertebral arch* is not quite so heavy and well marked in no. 886 as in Ia. The entire cartilage of the occipital region is somewhat lighter in structure. The lateral occipital eminence is not so prominent, nor is the cartilage here so thick.

The *condylar fossa* is represented upon the occipital cartilage by a depressed area, but as yet the superior articular process of the atlas does not lie far enough out to occupy it. It is relatively larger than that of the mature bone.

The *jugular process* is represented by a conspicuous transverse cartilaginous projection, which far overreaches the transverse process of the atlas below, in contrast to the condition in the adult.

There is a well-marked, though small, *processus ascendens*. It is a spheroidal mass of cartilage of rather young type which projects upward in the midline from the upper border of the tectum posterius—the central part of the supra-occipital cartilage. It is attached by a very short cartilaginous pedicle to the upper margin of the tectum. To either side, and closely associated with it, are the paired osseous spicules which represent the young interparietal bone, in their thin investment of condensed mesenchyme. The ascending process is thus attached to the unchondrified upper edge of the supra-occipital cartilage, already referred to, and which has been called by Bolk ('04) the 'Knorpelspange,' and other names.

Fawcett, in 1910, described this process in a human embryo of 30 mm., and homologized it with the *processus ascendens* of

the reptiles. This homology seems to me to be quite in order, but I would point out that the process in mammals does not bear the same relationship to the saccus endolymphaticus that it does in reptiles. Gaupp ('00) and Rice ('20) have described the saccus in lizards as lying just lateral to the ascending process, and Rice regards the process in the skink as affording protection to the saccus. In mammals, it need hardly be said, the saccus is far distant from this region, having accompanied the otic capsule in its evolutionary downward and outward rotation, and it follows that such a function on the part of the ascending process, if it ever existed, has become obsolete.

Mead, in 1909, made the same homologization for a small free nodule which he found in the pig's skull, just above the tectum posterius. In 1914 I described a free nodule of cartilage (with a very small grain of cartilage beside it) in the skull of Ia, and looked upon it as possibly representing the most superior cartilaginous mass described by Bolk ('04) in human skulls of about this stage and a little older. On account of the fact that the sections immediately posterior were missing from the specimen, I was unable to determine its relationship to the upper edge of the tectum posterius. It seems possible that these free cartilaginous masses may be vestiges of the tectum cranii anterius, a band which connects the parietal plates dorsally. Although this tectum is generally looked upon as rudimentary in man, Kernan ('16) has reported it as complete in his 20-mm. human embryo. Kernan has discussed the subject of the two tecta, lending support to the view of Levi ('00) that the anterior tectum disappears as the posterior appears. An interesting finding is reported by Fawcett ('18 b) in the embryonic skull of Weddell's seal. Two cartilaginous masses, lying side by side and showing slight signs of fusion with one another, appeared well forward in the membranous cranial vault, and are thought by Fawcett to belong to the anterior tectum.

I have found the ascending process in a number of embryos of the Carnegie collection, and am now engaged in a survey of this collection with the purpose of making a study of it. The process is represented in mesenchyme as early as the 21-mm. stage.

The work of Bolk ('04) upon the occipital region of human embryos has been much quoted in the literature, and has led a number of recent investigators into error because of a misinterpretation which it contains. Bolk used the van Wihje method to study the cartilage of this region, but the dyestuff (methylene blue) did not stain the calcified area of the supra-occipital cartilage representing the ossification center of the supra-occipital bone. Since this area did not give the tinctorial reaction for cartilage, and since he did not check his work with sections, Bolk concluded erroneously that it was membrane. His later stages show distinctly that this region does become ossified. He attempts to explain the occurrence of this 'membrane' by saying that rapid growth of the brain in this region has interfered with the development of cartilage; he fails, however, to account for the development of the 'Knorpelspange,' a band of cartilage which, as I have shown, represents the upper edge of the supra-occipital cartilage which has remained uncalcified, and which consequently stains blue in Bolk's preparations. It seems obvious that if the growth of the brain interferes with the development of cartilage in the region of Bolk's 'membrane,' it should certainly do so in the case of the 'Knorpelspange.' As a matter of fact, cartilage develops in the supra-occipital region quite early. I have found it at the 21-mm. stage in a human embryo. Calcification in the center for the supra-occipital comes on quickly. It should be said for one recent investigator (Fawcett, '10 b) that he was skeptical of this work of Bolk, saying (p. 306): "I must confess the appearances in his figures scarcely explain what is seen in this cranium."

It would seem that the upper edge of the supra-occipital cartilage, judging from Bolk's work, as well as my own, remains cartilaginous for a considerable time, and it may be that thus growth of the band in width is promoted through proliferation of the cartilage and subsequent invasion of it by the process of calcification, just as expansion of any endochondral center of ossification is brought about.

Bolk found, too, the region of the apex of the superior occipital incisure remaining cartilaginous, as shown by the staining with

methylene blue. The same reason may underlie the persistence of this cartilaginous mass. In one case, at least, Bolk finds that this paired mass of cartilage agrees in position with the later developing bones of Kerckring. These masses of cartilage, which Bolk thinks are in membrane, are doubtless really connected above with the calcified cartilage of the supra-occipital, and correspond probably to the processus descendens, as described in no. 886.

The *otic capsule* is well developed, the cochlear part showing the youngest type of cartilage, in accordance with the familiar order of chondrification. The walls are thin. A large *massa angularis* is present. The spiral septum is forming, but is far from complete. The cavity of the capsule was modeled as a solid, and the membranous labyrinth was also modeled. The latter is almost fully differentiated, and occupies but a small fraction of the available space within the capsule. The foramina are all large, and the edges are for the most part thin and of young cartilage.

The *malleus* and *incus* are separated by membrane, but do not show a distinct joint cavity. The *crus breve* of the *incus* is connected with the otic capsule by a small area of young cartilage. There is a small fragment of bone developing in the perichondrium of Meckel's cartilage, which represents the processus Folianus of the malleus, or goniale. It is as yet connected with the malleus only by membrane. The *stapes* has the well-known ring form, and the arciform base bends in the membrane filling the vestibular window. This membrane resembles pre-cartilage, and is not everywhere sharply marked off from the stapes. Anteriorly the latter is joined to the otic capsule by a narrow junction of young cartilage.

There was no *supracochlear cartilage*, as in Ia.

The *perilymphatic foramen* is large and shows evidence of the development of a partition which will separate it into its future parts; there is a small anterior and a somewhat larger posterior intraperilymphatic process.

The *styloid process* is directly attached to the crista parotica of the otic capsule by cartilage, whereas in Ia it was free from cartilaginous union with the capsule.

The *mastoid process* is a small free nodule in 886, whereas in Ia it was connected by cartilage with the otic capsule.

The *parietal plates* are relatively larger than in Ia. They have not, as yet, begun to be overlapped by the parietal bones. They are marked off from the occipital cartilage upon the cranial surface by the occipitoparietal groove. The cartilage along this groove is thinner than that of the plates above and below. Dorsomedially the parietal plate and occipital cartilage are separated by a distinct occipitoparietal notch, which, however, is not quite so deep as in Ia. There were no isolated nodules of cartilage in the vicinity of the upper margins of the parietal plates, as in Ia.

The *body of the sphenoid* is large, stout, and unperforated. From its side the *alar process* projects, and from the outer end of this process the temporal wing depends. From the caudolateral end of the alar process, which here is knobbed, projects backward the conical *alicochlear commissure*, whose pointed caudal end is confluent with the cochlear part of the otic capsule just below the pole of the latter. This commissure was not present in Ia, being represented by a short process, projecting backward from the processus alaris. The commissure subsequently develops into the lingula. The alar process, as Lewis ('20) points out, is largely taken into the body of the mature sphenoid, the carotid sulcus appearing upon its upper surface. The *carotid foramen* is closed by the alicochlear commissure in 886, and is much larger than the internal carotid artery which traverses its outer corner. Its posterior boundary is in part formed by a projection from the basisphenoidal cartilage which forms a union with the cochlear portion of the otic capsule, and represents the posterior petrosal process of the adult bone.

The *temporal wing* shows a distinct lamina ascendens and a pterygoid process. The foramen rotundum is complete, but the medial border is as yet thin and composed of young cartilage.

The *medial pterygoid plate* is a thin strip of bone, to the lower end of which is attached the cartilaginous hamular process. The line of junction between the two is not well marked.

The *orbital wing*, larger than the temporal, is thin. Its posterior root is stout and rounded, but the anterior root is of precartilage, is very slender and is evidently just forming. From the caudal edge of the anterior root there passes back a slender strand of precartilage, the fundament of the *prechiasmatic commissure*, which was represented in Ia in cartilage, and was much stouter. This commissure cuts off the small prechiasmatic foramen from the larger optic foramen. The prechiasmatic foramen is relatively larger in 886 than in Ia. Contrary to my former statement (Macklin, '14), it may persist in adult skulls—at least in those of younger age. I have recently noted these foramina in the sphenoid of a young adult in the osteological collection of Johns Hopkins Medical School. In several older skulls it was not present.

From the edge of the presphenoid, just in front of the posterior root of the orbital wing, there projects outward the *ala hypochiasmatica*. According to Kernan ('16), this structure represents a separate center of chondrification for the ala orbitalis. In 886 it is of a young type of cartilage, edged with precartilage, and is noticeably less developed than in Ia.

Each orbital wing has a well-marked dorsolateral process, which turns upward a little, as well as outward and backward. With the limbus sphenoidalis these two points make an angle of 131° , contrasting with the average of the corresponding angles taken from four mature skulls, which was 150° . Thus there is a flattening out of the orbital wing with subsequent development, associated, no doubt, with the growth of the brain in this region.

The anterior part of the orbital wing is bordered medially by the orbitonasal fissure, but laterally it is continued forward into the spheno-ethmoidal cartilage, which is connected with the anterior part of the ectethmoid by the spheno-ethmoidal commissure.

The *nasal septum* is not so stout as that of Ia, particularly along the lower border. It presents no superior paraseptal cartilages, as in Ia. The *cartilages of Jacobson* are not so far developed as those of Ia, but the different parts can be made out in them. They are of young cartilage and precartilage, and

the medial plate is connected anteriorly with the ventrolateral process and with the nasal septum. They are situated considerably below the level of the *vomeronasal organ*.

The *vomeronasal organ* was modeled, and is fusiform in shape. It is connected with the nasal cavity through a developing duct in which a definite lumen has not yet formed. The main portion of the organ suggests a coiled duct, but the lumen is not definite. The septum, medial to the organ, is not hollowed out, as in Ia.

The *mucous membrane* covering the septum is fairly flat, and shows an indistinct wide and low elevation which runs almost parallel with the upper border.

The *ectethmoid* is a thin, irregularly shaped plate of cartilage, upon the inner surface of which are to be seen the representatives of the future conchae. The superior concha is as yet of precartilage, almost entirely, and is wide, low, and rather indefinite. The middle concha is well marked, though not so well as in Ia, and it does not show such a definite continuity caudally with the maxilloturbinate as in Ia.

In the middle meatus there is a long slender process which may represent the *uncinate process* of the adult. It is altogether of precartilage and corresponds to the cartilage of the middle meatus of Ia, where it was a small nodule of young cartilage attached by a pedicle of precartilage to the ectethmoidal wall. The region of its attachment corresponds outwardly to the location of the posterior maxillary process.

The *inferior concha* is the largest of the three, and is the lower portion of the ectethmoid which has been bent upward and inward. Anteriorly it slopes downward, but posteriorly it projects almost directly inward, and the inner edge, covered with young cartilage, turns downward. Anteriorly it is bounded by the posttransverse incisure. The medial extremity of the maxilla, underlying the paraseptal process, makes of the incisure a foramen, leading into the inferior nasal meatus. There is a small cavity representing the spheno-ethmoidal recess, and one representing the superior nasal meatus. Anteriorly the agger nasi is represented very indefinitely by a low eminence.

The roof of the nasal capsule is very incomplete, the cribriform plates having only begun to form, and their loci being represented by gaping foramina; posterior to these there is a fenestrated covering of young cartilage.

The outer wall presents posteriorly the familiar flattened planum antorbitale which bears, near the upper surface, a very small paraethmoidal process, directly attached to the wall. In Ia this was a free nodule. The anterior portion of the plate shows several eminences. The superior nasal prominence is low and makes no corresponding concavity upon the inner surface. The middle prominence, or *Sakterwulst* of Voit ('09), is very conspicuous. The inferior prominence is represented in the interior by a hollow, and is continued backward into the long and slender paraseptal process. Upon the lower edge of the prominence there is a small tubercle, the superior alar process which appears to be taken into the lateral crus of the greater alar cartilage in later stages, according to the researches of Remke ('13), as stated in the atlas of Peter ('13). The paraseptal process, too, is shown by the same investigator to become separated later on into fragments to form the lesser alar cartilages. This process represents part of the anterior transverse lamina of the lower forms, and it is of interest that the lesser alar cartilages of the nose are derived from it.

There is a distinct posterior nasal prominence which leads backward, outward and downward as a ridge to join with the sharp lower edge of the planum antorbitale. The process is partially cut off by a distinct cleft from the posterior maxillary process, which is edged with a young type of cartilage, and which shows projecting forward from it the very slender spicule of osseous tissues representing the lacrimal bone. Lateral to these structures is the nasolacrimal duct. There is a distinct club-shaped paranasal process, the narrow end being attached to the capsular wall, while the blunt anterior end, which also points downward, lies just lateral to the nasolacrimal duct. In Ia it was free. In subsequent ossification this region is apparently included in the lacrimal bone.

An interesting structure in 886 is the *anterior cupular process*. It is a very long and slender hook-like process of precartilage. It is directly attached to the projecting anterior margin of the ectethmoid which forms the front of the anterior naris. It is crescentic in shape, the concavity looking outward, and containing the epithelial plug of the anterior naris. At the summit of the curve the two processes are very close together, and they are also quite close to the nasal septum here. The posterior end of the process is very sharp and turns outward. Rehmke ('13), in a somewhat later stage, has found this process represented in cartilage (Peter, '13), and in the 275-mm. stage (sitting height) he has noted that it joins with the ventrolateral process to form the medial crus of the greater alar cartilage. It is of interest to find the medial crus represented so early in precartilage.

The *nasolacrimal duct* together with the nasolacrimal sac and the lacrimal ducts were all modeled. There is as yet no distinct lumen. The duct is quite slender and has a curved course. The sac is not at all a marked dilation. The lower end of the duct is applied closely to the epithelium of the inferior meatus (which is an uncleft plate in this region), but does not as yet open into the nasal cavity.

The position of the lower end of the nasolacrimal duct is far behind that of the corresponding structure in the lower forms. As an example (taken from many), we may cite the condition in the cat (Terry, '17), where the duct courses forward, laterally to the anterior transverse lamina (represented in homo by the paraseptal process), and opens into the nose in front of this lamina through what is really an extension of the anterior naris. We have noted in Ia that there is no cartilaginous connection between the paraseptal process and the nasal septum, and the same condition obtains in 886, and it would seem that this structure, in man, does not develop. Thus it is not impossible that the retrogression of the lower end of the nasolacrimal duct has been associated with the disappearance of the anterior transverse lamina. When this lamina is removed there is no cartilaginous impediment to the caudal migration of the duct, and its

consequent shortening. The shortening of the duct, too, has doubtless been associated also with a shortening in the length of the nose.

The *notochord* was modeled in relief. Its course is very similar to that described by Huber ('12) for his embryo J, no. 47, 32 mm., shown in his figure 10. It presents varicosities in the region between the tip of the dens and the basioccipital cartilage. In traversing the basioccipital it runs through the anterior end of the preossification center, and is here much attenuated—indeed it shows a break in continuity just at its point of exit from the plate. It comes into close relationship with the pharyngeal bursa in the well-known manner, and here presents more varicosities. After traveling close to the pharyngeal epithelium for some distance, it again enters the cartilage of the basal plate to terminate in the basisphenoid just below the root of the dorsum sellae.

Several of the cranial nerves were modeled, including much of the V and VII, and some of the IX, X, XI and XII. The related ganglia, as the semilunar, geniculate, vestibular, cochlear, sphenopalatine, jugular or glossopharyngeal and vagus petrous and nodosum, were modeled. The internal carotid artery, auditory tube and tympanic cavity, and stapedius muscle and tendon are among other structures modeled. The relation of the skull to the brain and to the external form is shown in a profile reconstruction.

Membrane bones. The membrane bones are all present excepting the nasal. Some of them are very small, as the tympanic interparietal, lacrimal, goniale and medial pterygoid plate. The maxilla is of interest in that it presents a groove upon the palatine surface which leads up to the tip of the frontal process. This groove represents the incisive suture, separating the maxillary and the premaxillary elements, as recently described by Felber ('19); (abstract by Schultz, '20). The upper end of this suture is imperfect, representing the region latest to close.

The cartilaginous branchial arch skeleton was modeled. The *hyoid cartilage* has a lesser cornu of young cartilage connected by a membranous strand with the styloid process. There is a

perforation in the *thyroid cartilage* which is closed below by precartilage. The *cricoid cartilage* bears the small *arytenoid cartilages*, which are very small nodules of young cartilage enclosed in a thick layer of condensed mesenchyme which shows several of the characters of the adult cartilage, and which is directly continuous with the mesenchyme of the cricoid cartilage. The tracheal rings are rudimentary as yet.

A full description of this skull with illustrations will appear at an early date in the Contributions to Embryology (Vol. 10), published by the Carnegie Institution of Washington.

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Resumen por el autor, George B. Wislocki.

Nota sobre el comportamiento del azul tripan inyectado en el huevo de gallina en vías de desarrollo.

Una solución coloïdal, el azul tripan, fué inyectada bajo precauciones asépticas en huevos durante el undécimo día de incubación. El pequeño orificio abierto en la cáscara, a través del cual se inyectó, fué cementado, volviendo a colocar el huevo en la incubadora. Después de dos días se abrió el huevo examinando el embrión. El colorante fué inyectado en la cámara aérea, saco vitelino, saco alantoideo, saco amniótico y en el mesodermo del alantoides. En cada una de estas regiones se comporta de modo diferente. La membrana de la cáscara que rodea a la cámara aérea le absorbe rápidamente. Escapa desde el saco vitelino a través de las células epiteliales endodérmicas que forran el saco interiormente, pasando al mesodermo subepitelial, donde es absorbido y almacenado por las células mononucleares que rodean a un plexo de vasos vitelinos.

Cuando se inyecta en el saco alantoideo el colorante no es absorbido. Cuando se inyecta en el saco amniótico tiñe al epitelio amniótico y también penetra en el tracto gastrointestinal del feto por la boca. Cuando se inyecta directamente en el mesodermo de la membrana fetal se difunde en la corriente sanguínea del feto tiñendo vitalmente al embrión.

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NOTE ON THE BEHAVIOR OF TRYPAN BLUE INJECTED INTO THE DEVELOPING EGG OF THE HEN

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THREE FIGURES

Attempts have been made by several investigators to determine the fate of dyes injected into the egg and their effect upon the embryo, but the experiments, though yielding a few results, were very brief.

Zaretsky ('10) injected trypan blue, trypan red, neutral red, methylene blue, fluorescein and eosin into developing eggs; and obtained results of greatest importance with the first two. He injected 0.5 to 1 cc. of one of these dyes into the air chamber of the egg, and after a period of days noted a slight staining of the amniotic fluid, but never any staining of the embryo itself. In other instances, after injecting the dye into the outer wall of the allantois, he observed a faint staining of the entire embryo, fetal membranes, amniotic fluid, and albumin sac. He stated that of the tissues of the embryo the kidneys were the most deeply stained, indicating a rapid excretion of the dye by that channel. Granules of dye could not be identified microscopically in the tissues.

Gräper ('12) reported the staining of early chick embryos with neutral red, trypan blue, and trypan red, but nothing more than diffuse staining of membranes and embryo alike was observed.

The most valuable results following the injection of dyes into the developing chick so far reported are those obtained by Bakounine ('95). She injected indigo carmine intravenously into a series of chicks ranging in age from three to fifteen days; excretion of the dye by the tubules of the wolffian bodies was demonstrable in the entire series.

The present paper is a preliminary account of some observations upon the behavior of trypan blue injected into developing eggs. The experiments were undertaken in spite of the failure of previous investigators to make any significant observations with vital dyes in the chick. It was hoped that by injecting the dye into different regions of the egg, some knowledge concerning the functions of the fetal membranes, and possibly a successful vital staining of the embryo, might be obtained.

Chicks of eleven days' incubation were used. Trypan blue, which forms a colloidal sol, was made up in 1 per cent strength in sterile distilled water. The injections were made with a syringe bearing a 26-gauge needle, 1 inch in length, through a nick in the shell, the opening being no larger than necessary to admit the needle. The trypan blue solution (0.2 cc.) was injected into each egg, whereupon the needle was withdrawn and the tiny opening in the egg-shell sealed with a drop of melted paraffin. The eggs were then returned to the incubator to be opened at the end of forty-eight hours—the thirteenth day of incubation.

By this method, of course, it cannot be determined exactly at the time of injection into what region of the embryonic membranes the trypan blue has been injected. If, with a general knowledge of the orientation of the fetal membranes and embryo, a number of eggs are injected, each locality which it is desired to inject will be reached in a certain number of eggs. This is made plain by reference to the diagram shown in figure 1, which illustrates conditions in the egg about the eleventh day. Thus it will be seen that by stabbing a needle into the egg, with a knowledge of the orientation of the structures within, one will in many instances inject with precision the yolk sac, allantoic sac, amniotic sac, etc. Orientation is made easier if one remembers that, after the egg has lain undisturbed in the incubator for a few hours, the embryo floats to the upper surface; hence the amniotic sac is more readily injected from above, and the yolk-sac from the side or below. Suffice it to say that in a series of five dozen eggs, injected as described above and killed on the thirteenth day of incubation, all of the selected points have been successfully injected. A description of the findings in each of the groups follows.

Injection into the air chamber. The air chamber is a cleft between the two layers of the shell membrane. The membrane is composed of matted fibers of organic substance which cross one another in every direction. When trypan blue is injected into this chamber it is rapidly absorbed by the shell membrane, much as it would be by blotting paper, staining it a deep blue. The adjacent structures beneath the shell membrane, namely, the chorion and allantois, as well as the embryonic fluids, remain

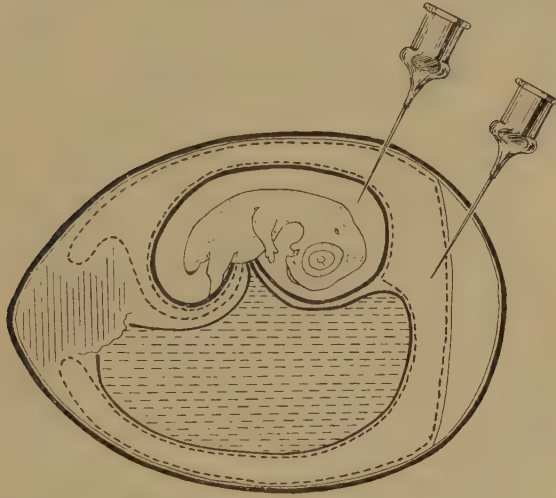


Fig. 1 Schematic representation of a chick approximately on the tenth day of incubation, showing the technique used in injecting different localities of the egg.

unstained and consequently also the embryo itself. When the shell membrane is removed, the inner surface of the shell is frequently found colored in the neighborhood of the air chamber.

Injection into the yolk-sac. On injecting trypan blue into the yolk-sac the dye remains somewhat localized in the yolk in the neighborhood of the injection, lending a greenish appearance to the yolk. The trypan blue, together with the yolk, is absorbed by the endodermal epithelial cells lining the interior of the yolk-sac. The dye imparts a greenish-blue coloration to the wall of the yolk-sac. Microscopically, a greenish, diffuse coloration

of the cytoplasm of the yolk-sac epithelium is visible. The dye enters the epithelium most abundantly in the region of the area vasculosa, where the wall of the yolk-sac is thrown into numerous folds covered by large columnar cells which possess swollen ends and the cytoplasm of which is closely filled with droplets of yellow substance.

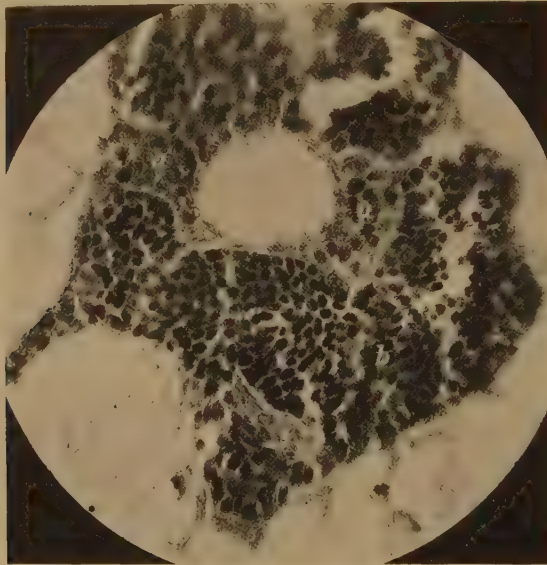


Fig. 2 Photomicrograph of the wall of the yolk-sac of a thirteen-day chick, showing a portion of the area vasculosa. A group of cells which ingest vital dye (a) is seen adjacent to a blood vessel. Other groups of cells (b) are hematopoietic and do not phagocytize dye.

The dye eventually penetrates the basement membrane upon which the endoderm rests and reaches its final destination in groups of cells surrounding the rich venous network in the yolk-sac wall. These cells are closely associated with groups of hemoblasts which invest the blood-vessels. The former are round or polygonal and possess a central or slightly eccentric round nucleus. Their cytoplasm absorbs the vital dye and stores it in the form of closely packed blue granules (fig. 2). These cells appear to be the ultimate destination

of the dye injected into the yolk-sac, as no evidence of its further passage is discoverable. The endothelial cells lining the blood-vessels remain unstained and, since the embryo itself does not become vitally stained, it is unlikely that any of the dye escapes into the vitelline vessels. It is of interest to note that in spite of the fact that throughout incubation the yolk-sac possesses a connection through its stalk with the intestine, none of the dye injected into the sac enters the intestine through this opening.

Injection into the allantoic sac. When trypan blue is injected into the allantoic sac it mixes uniformly with the allantoic fluid, coloring the latter dark blue. The dye does not escape from the allantoic sac—a fact which can be demonstrated in several ways. If the discolored allantoic fluid is drained off and the sac washed out with physiological salt solution, the allantoic membrane is found to be unstained. The fact that the embryo and all its membranes are unstained confirms the view that no dye has escaped from the allantoic sac. Furthermore, microscopic examination of the wall of the sac reveals the absence of the dye in the delicate, flat polygonal cells which cover its surface, or in the layer of star-shaped cells just beneath the surface. These observations accord well with the view that the allantois is a reservoir for the excretory products of the mesonephros and metanephros.

Injection into the amniotic sac. Trypan blue mixes uniformly with the amniotic fluid. The amniotic membrane becomes pale blue due to the presence of dye, which in some instances is visible under the microscope as blue dust in the delicate, flattened epithelial cells lining the surface of the membrane. The stomach and intestines invariably contain dark blue stained mucoid fluid which indicates that the amniotic fluid is swallowed by the embryo. Stained fluid is also observed in the lumen of the trachea and primary bronchi. A pale blue staining of the entire embryo is frequently observed, but the quantity of dye is insufficient to make it visible in the tissues when examined under the microscope.

Injection into the mesoblast of the allantois. When trypan blue is injected into the mesoblastic tissue uniting the allantois with the chorion and amnion, vital staining of the embryo results. This is due, no doubt, to the easy access which the dye has to the network of allantoic vessels through which it is soon conveyed to all parts of the embryo.

The membranes and integument of the vitally stained embryo present a light blue aspect. The yolk remains unstained, as does also the shell membrane and the shell itself. The depth of color in the various organs of the embryo proper varies: the central nervous system is unstained, the arteries are conspicuously blue, the lungs pale blue, the liver is greenish blue, the spleen reddish blue. The wolffian bodies are by far the most deeply stained organs; the metanephros is appreciably blue.

Microscopic examination of the tissues reveals the presence of trypan blue in granular form in several of the organs. In the thirteen-day-old chick it is found in most abundance in the wolffian bodies. Here it occurs in the shape of numerous tiny granules in the epithelium lining the uriniferous tubules (fig. 3). As might be expected from our knowledge of the distribution of vital dyes in the adult renal apparatus, no dye is discovered in the glomerular capsules. In the metanephric tubules, which at this period are already quite distinct, only traces of the dye are visible. It would appear, then, that at this period of development the wolffian body still serves as the main pathway of excretion, though regressive changes in the tubules are already plainly visible.

The second organ in which trypan blue appears in abundance is the embryonic liver. The dye is found microscopically in nearly all the endothelial cells lining the sinusoids and the terminal branches of the portal vein. In the liver cells themselves no particles are visible.

The spleen, which in the thirteen-day-old chick is a small round organ approximately 2 mm. in diameter, contains traces of trypan blue which appear to be within cells lining the vascular channels and occasionally within mononuclear cells lying free within the sinuses.

Nowhere else in the tissues of the embryo is trypan blue abundant, although traces of dye are encountered not infrequently in the connective tissue in cells resembling clasmatocytes. That there are cells in the connective tissue of the chick capable of phagocytosis is best shown by examining the mesoderm at the site of injection of the trypan blue into the wall of the allantois.



Fig. 3 Photograph of a drawing of the wolffian body of a thirteen-day-old chick, showing a glomerulus with the beginning of a tubule. The black dots represent granules of vital dye.

Here numerous mononuclear cells, some of them round, others irregular in outline, are encountered with dye granules within their cytoplasm.

The behavior of trypan blue in the thirteen-day-old chick seems to be of sufficient interest to warrant an extension of these observations to other stages of development. The findings described suggest paths of investigation which it be might of interest to pursue. For instance, an investigation of the function of the mesonephros and the permanent kidneys as excretory organs

during embryonic life would be of considerable value. A study of the absorption of vital dyes from the yolk-sac at different stages would likewise furnish us valuable knowledge concerning the physiology of absorption in the embryo. The method used in these experiments will, however, never be applicable to chicks under four or five days. In these younger stages direct observation of the living developing chick under the microscope will always prove more satisfactory.

SUMMARY

A series of experiments is described upon the behavior of a vital dye, trypan blue, injected into the developing egg of the hen, and the technique of injection is given. In the vitally stained chick granules of dye are found in cells of the mesonephros, metanephros, liver and spleen.

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Resumen por el autor, H. E. Jordan.

Nota sobre la citología del cuerpo pineal de la oveja.

Las células de "interneuroglia" del cuerpo pineal de la oveja de cuatro a ocho meses se caracterizan, (en el tejido fijado durante cuatro semanas en una solución al 2 por ciento de ácido ósmico), por la abundancia de pequeñas mitocondrias esféricas y un número variable de glóbulos lipoides más grandes. Entre la cantidad de mitocondrias y la de gotitas de lipoide existe una relación recíproca bastante aparente. La existencia de una relación genética entre ambas estructuras, como se ha indicado, sigue siendo una cuestión pendiente de solución. Los glóbulos de lipoide constituyen la única prueba citológica indicadora de una actividad secretora. Las células carecen aparentemente de centrosoma y se dividen solamente por amitosis durante este periodo. Ciertas transformaciones, tanto fisiológicas como mecánicas, en las grandes gotas de lipoide producen estructuras que simulan un retículo de Golgi y un trofospongio.

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A NOTE ON THE CYTOLOGY OF THE PINEAL BODY OF THE SHEEP

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ONE PLATE (ELEVEN FIGURES)

In an earlier paper on the histogenesis of the sheep's pineal body, I ('11) stated the conclusion that 'There is no clear histologic evidence indicative of a glandular function. The sole cytoplasmic granules are melanic and probably have only ancestral significance' (p. 269). In a foot-note (p. 265) I recorded my experience with the Altmann osmic acid-potassium bichromate mixture, employed in an effort to reveal mitochondria. This technic disclosed the presence of very numerous deeply staining irregular granules. This granular character of the cytoplasm was interpreted as an artifact. But the Altmann technic preserved also certain large lipoid globules of variable size, many of which were in process of disintegration into 'masses of very small black granules.' These lipoid bodies I interpreted at the time as probably indicating 'intracellular degenerative changes, and not mitochondrial or secretory in nature.' My general conclusion was that 'If the pineal body of the sheep subserves an important physiologic function, this is probably active only during the first eight months of post-natal life' (p. 269).

Tilney and Warren ('19) conclude that the histologic evidence indicates that the pineal body of vertebrates should be classified as a glandular structure (p. 225). They state that 'The final conclusion to be drawn from the histological evidence in the epiphyseal complex of vertebrates would seem clearly to indicate that this structure of the pineal region possesses a pluripotentiality whose fundamental, inherent tendency is in the interest of glandular differentiation' (p. 226). They seek support, in part, for the conclusion of a glandular nature of the pineal body

from the occurrence of mitochondria in neuroglia. Granting that the cell constituency of the epiphysis is, in major part, neuroglia, as claimed by Dimitrova ('01) and by Jordan ('11), they state that 'this admission would not wholly invalidate the idea that the structure is glandular in nature, for, according to the most recent researches of Nageotte and Mawas, neuroglia cells contain mitochondria and hence, according to these investigators, should be considered as glandular elements' (p. 225).

Recently Portella ('20) has described and illustrated certain lipid bodies in the anterior lobe of the dog's hypophysis variously fixed and stained by methods designed for the demonstration of fat, which appear practically identical with those discernible in the epiphysis of the young sheep in tissue treated according to the Altmann technic. The presence of these lipid bodies he interprets as indicating a fatty secretion on the part of the hypophysis.

Mawas ('10) describes in the ependyma and general neuroglia of the central nervous system mitochondria, secretion grains ('grains de ségrégation'), and lipid inclusions. The mitochondria are variously designated 'chondriocontes' and 'filaments mitochondriaux.' The predominating type of mitochondrion seen by Mawas was apparently of the rod or bacillary type. The illustration given by Nageotte ('10) of neuroglia in the gray matter of the rabbit and guinea-pig, treated according to the Altmann technic, shows a small spheroidal type of mitochondria. Besides mitochondria, Nageotte describes also two other inclusions: 'grains vacuolisés' and 'grains de sécrétion.' He claims that these three categories of granules are genetically related, the minute spherical mitochondria passing through a stage represented by a large granule with a clear center to a slightly smaller fuchsinophil secretion granule. These investigators base their interpretation of a secretory function on the part of the neuroglia, not upon the occurrence of mitochondria, as the quotation from Tilney and Warren would seem to suggest, but rather upon the presence of other cytoplasmic inclusions, namely, lipid globules and so-called secretion granules. The question of the genetic relationship between mitochondria and

the presecretion granules of glandular cells lies outside the scope of the present communication.

In my earlier paper I traced the histogenesis of the sheep's pineal from its origin from the ependyma to its definitive condition as a mass of essentially neuroglial constitution. During the earlier postnatal period two types of cells were distinguished: the so-called interneuroglia cells and the neuroglia cells. The latter represent simply more fully differentiated neuroglia elements. As compared with the generally polyhedral interneuroglia cells, the definitive neuroglia cells of the epiphysis are flattened, irregularly stellate or fusiform, with oval nucleus and smaller amount of cytoplasm. After the first year the interneuroglia cells become practically indistinguishable from the general mass of neuroglia tissue. The present investigation has reference only to the interneuroglia cells.

In view of the fact that mitochondria have been demonstrated in the neuroglia of the brain and cord, it would seem legitimate to infer that the younger neuroglia ('interneuroglia') cells of the pineal body also contain mitochondria. But I am not aware that anyone has actually described mitochondria in the cells of the pineal. However, the occurrence of mitochondria in the cells of the pineal body cannot be interpreted as indicative of a secretory function. Mitochondria have now been demonstrated in practically every kind of cell, non-secretory as well as secretory. In other words, the presence of mitochondria is not a specific index of secretory activity. Regarding the lipoid bodies demonstrable by the Altmann technic, the case is different. The question remains whether these bodies, originally interpreted as a coincidence of the degenerative processes in the pineal, may not actually indicate a secretory rôle.

With these points chiefly in mind, I undertook a reinvestigation of the cytology of the sheep's pineal of the first year. I desired mainly to determine whether the finer cytoplasmic granulations of the cells as revealed by the Altmann technic might not be in fact modified mitochondria; and further, if possible, what is the relation of the mitochondria to the larger lipoid bodies. But as the investigation progressed it involved

other cytologic details of the pineal cells, including thus, besides a consideration of mitochondria and the larger lipoid bodies, also a search for evidence of a Golgi apparatus, a trophospongium, specific secretory granules, and a centrosome.

In a recent study of the cytology of the hemogenic giant-cells of the red bone-marrow of the rabbit and guinea-pig I ('21) succeeded in disclosing both mitochondria and a Golgi apparatus in the same cells by a slight modification of the Kopsch technic. I decided, therefore, to employ the same technic in a reinvestigation of the pineal body of the sheep. The following description is based chiefly upon material obtained from sheep ranging in age from four to eight months. Comparison with testicular tissue successfully fixed in the same way indicates that the fixation of the cells of the pineal is similarly satisfactory. The tissue has a generally clear golden-yellow color, with the cytoplasmic inclusions stained a deep brown or black. Unsuccessful preparations have a quite different appearance; either a straw color or a deep brown color, with a somewhat muddy appearance and a granulated character. The best-preserved area is some distance below the surface of the piece of tissue. The surface and center have generally the straw color and the muddy appearance, respectively, indicated above for poorly fixed tissue. It seems legitimate to infer that cytologic elements (except the centrosome) not revealed by this technic in this tissue do actually not occur; for this technic reveals mitochondria, Golgi net, and other lipoid bodies in certain other tissues.

The tissue was obtained from the abattoir and fixed either within thirty minutes after slaughter of the animals, or after the heads had been on ice for from one to two hours. No essential differences were noted in the results following the two procedures. Pineal bodies of sheep of from four to eight months are essentially identical as regards the cytology of the interneuroglia cells. The distinctive melanic granules of the prenatal stages have generally disappeared by this period, and are nowhere sufficiently abundant to confuse the interpretation.

For purposes of comparison, portions of the same pineal body were in every case fixed also in a 10 per cent solution of formol,

in 80 per cent alcohol, and in the Zenker-formol mixture of Helly. The tissue was imbedded in paraffin, sectioned at from 3 to 5 μ , and stained with hematoxylin and eosin. The Helly-fixed tissue was stained also with Wright's stain and with the Giemsa mixture. The latter procedures were used in an effort to disclose possible secretory granules other than lipoid.

With none of the techniques employed could a centrosome be disclosed. Nor could any cells be found in process of mitosis. Occasional cells occur with two nuclei, more or less closely apposed, indicating amitotic proliferation. In fetal and early post-natal life these cells divide at least largely by mitosis, as described in my earlier paper. It would seem that the later growth of the pineal body during the first year is largely dependent upon amitotic divisions. Lack of centrosome and amitosis may be causally related. In tissue prepared by the Kopsch technic a structure occasionally appears simulating a centrosphere with an included centrosome (fig. 4). This centrosphere simulacrum will be discussed below, where its deeply staining periphery, simulating a Golgi apparatus, will also be considered and interpreted.

Careful study of the preparations leaves little doubt concerning the presence of mitochondria in the pineal cells. They are generally of the granular, spheroidal type (figs. 1 and 2). Occasional bacillary and annular types occur. The former occur more generally in the more nearly spherical cells with a narrow shell of cytoplasm, apparently the younger cells; the latter occur mingled with the granular forms in the larger more irregular cells with a wide shell of cytoplasm, the more differentiated cells. Mitochondria are discernible in all of the cells, neuroglia as well as interneuroglia. The number of mitochondria varies greatly in different cells. They are generally relatively more abundant in the smaller polyhedral cells. There is also a fairly definite numerical correlation between the mitochondria and the lipoid globules; when the globules are abundant the mitochondria are relatively few, and vice versa.

The lipoid globules range in size from minute bodies to relatively enormous spheres (figs. 3, 6 and 7). The most difficult point in this connection concerns the line of demarcation between

a spherical mitochondrion and a small 'lipoid body.' This matter will be discussed after a further description of the larger lipoid bodies. The latter are apparently readily soluble, for they have disappeared in all of the specimens preserved in alcohol, formol, or Helly's fluid. They may occasionally be discerned as rather pale yellowish shadows in some of the cells in certain areas of tissue fixed with the strong solution of Flemming. Similarly, the smaller granules, presumably mitochondria, can occasionally be seen in cells of the Flemming-fixed tissue. The vacuolated condition of the cells of the pineal, fixed in other than osmic acid solutions, is undoubtedly largely the result of the solution of these lipid constituents by the fluids employed in these technics.

The larger lipid spheres undergo several distinct changes, all apparently, however, leading to the same end result, namely, liquefaction and disappearance. The large lipid globules, as previously described for tissue treated with the Altmann technic, may fragment into a mass of minute spherical granules. More generally, in the tissue used in the present investigation (preserved according to the Kopsch technic), the globules become pale (dissolved) centrally and appear as more or less clear spheres with a deeply staining periphery (fig. 10). The latter represents the more resistant lipid hull of the globule. This may be more or less regular, simulating a small Golgi apparatus (figs. 4 and 5). The interpretation of a Golgi apparatus is suggested especially when the clear central area contains one or several deeply staining granules, thus simulating a centrosphere with a centrosome. Almost as frequently the globules appear to dissolve peripherally, thus leading to a condition where a clear circular area includes centrally a deeply staining granule of variable size. Occasionally the globule dissolves in such manner as to have for a while both a deeply staining periphery and a central granule (fig. 9).

Frequently also one sees an entirely clear sphere surrounded by an envelope of small spherical granules, generally arranged in single file. The latter again suggests a localized Golgi apparatus in process of formation through fusion of mitochondria (figs. 5 and 8). A confident interpretation of these varying

conditions is difficult. As stated above, where the large lipid bodies are numerous, the small spherical granules (mitochondria) are relatively infrequent. The condition of the clear sphere with peripheral granules appears to represent two different things: In one case it represents a lipid globule which has fragmented into small granules, all of which have disappeared except the more peripheral ones. More generally, however, I believe that it represents a large globule which has become liquefied (or at least chemically changed so as to lose its former reaction to osmic acid) and has, during the enlargement involved in its growth or liquefaction, compelled a mechanical arrangement of the neighboring granules about its periphery. No doubt also such arrangement under pressure may subsequently effect a coalescence of the involved mitochondria and produce a clear sphere enveloped by a dark shell, thus again giving the appearance somewhat of a small Golgi apparatus.

Other than the complex above described as simulating a Golgi apparatus, there is no indication of such a structure in my preparations. Assuming that the interpretation as a Golgi-net simulacrum is correct, and that the Kopsch technic is specific for the Golgi net, we must conclude that the cells of the pineal of postnatal stages lack a genuine Golgi apparatus. Recalling that the Golgi net commonly forms first in the neighborhood of the centrosphere, the absence of a Golgi net in these cells may be correlated with the absence of the centrosome and the consequent amitotic mode of proliferation.

The cells of the pineal, whether fixed with alcohol, formol, Helly's fluid, Flemming's fluid, Altmann's fluid, or the Kopsch fluid, have a vacuolated cytoplasm. This vacuolization is least extensive in tissue fixed by the Kopsch technic. What vacuoles appear should probably be interpreted as the negatives of functionally liquefying globules. Such interpretation is correct no doubt also with regard to some of the vacuoles in tissue fixed with the Flemming and Altmann fluids. Some of the vacuoles no doubt are the negatives of globules dissolved by the fluids employed in these technics. The latter interpretation applies almost exclusively for the other technics. The vacuolization is

most extensive in alcohol-fixed tissue. Aside from the vacuoles, the cytoplasm of the cells preserved in 10 per cent formol is most nearly homogeneous. None of the technics employed disclose a specific granulation other than the mitochondria and the lipid bodies.

This brings us to the question of the relationship between the smaller spherical granules, interpreted as mitochondria, and the larger lipid bodies. Four obvious possibilities present themselves: 1) that the mitochondria become transformed into the larger globules; 2) that the mitochondria are derived by fragmentation from the larger lipid spherules; 3) that the mitochondria and lipid spherules sustain no genetic relation to each other, and, 4) that the entire granulation, including the most minute as well as the largest lipid bodies, are the same thing at different stages of growth, that is to say, that all the deeply colored bodies in the Kopsch-preserved tissue are either all mitochondria or all lipid bodies of different sizes. My preparations would seem to supply data for the partial support of any one of these interpretations. For example, the fact that one can find no line of demarcation, either on the basis of size or staining reaction to osmic acid, between the assumed mitochondria and the larger lipid bodies, might be used to support either the conclusion that they are one and the same thing or that the latter developed from the former. Furthermore, the reciprocal numerical relationship between the mitochondria and the lipid globules could be cited in support of the origin of lipid bodies from mitochondria, or vice versa. The fact that the larger lipid globules break up into smaller granules, indistinguishable from the assumed granular mitochondria, could also be used to support the contention that mitochondria are derived from lipid globules. And, finally, the mechanically imposed orderly arrangement of the smaller granules (mitochondria) about the growing and liquefying large clear spherules could be cited to sustain the argument of a relative independence of these two cytoplasmic constituents.

Similar difficulties have presented themselves in numerous other studies dealing with mitochondria and fatty globules.

Positive statements regarding a mitochondrial origin of fat are made, for example, by Schreiner and by Russo. Schreiner ('15) concludes that in the lipoblastic mesenchyme cells of the Myxine embryo fat globules are transformed mitochondria. Russo ('09) claims to be able to increase the mitochondrial content, and secondarily the deutoplasmic content, of the ova of rabbits by feeding or injecting lecithin. He believes that mitochondria are transformed into yolk globules. Coghill ('15), on the contrary, thinks he can see in the living tissue, and in sections, of amphibian embryos a granular transformation of the surface layer of certain deutoplasmic globules ('alpha globules') into mitochondria. The two elements, 'alpha bodies' of yolk globules and mitochondria, are said to react identically to certain assumed specific stains for mitochondria (e.g., janus green and Bensley's acetic-osmic-bichromate method).

The chemical relationship between mitochondria and certain fatty bodies is undoubtedly very close. Cowdry ('18) also has called attention to a general 'distinct reciprocal relationship between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is much fat, and *vice versa*' (p. 82). At another place Cowdry says that '*Large spherical* mitochondria are usually on the border-line between true mitochondria and lipid droplets. They represent a stage in the transformation and may be found in any tissue where the change is taking place' (p. 69).

The tissue under investigation does not seem to me sufficiently favorable as a basis for a profitable further discussion of the question of the relationship between mitochondria and lipid globules. The only thing that can be said positively regarding this matter with the data at hand is that in the so-called inter-neuroglia cells of the pineal body of the sheep of the first six months there occur, as revealed by the Kopsch and Altmann technics, small bacillary and spherical granules similar to mitochondria of other tissues, and that among these occur large lipid droplets, and that the two are reciprocally related in amount.

Apparently the two elements sustain a close genetic relationship, but the proof of such relation is far from complete. It

may be emphasized, however, that neither of these structures can be interpreted as an artifact. In the first place, the fixation, as compared with other tissues similarly treated and where both mitochondria and Golgi apparatus appear sharply, seems perfect. Furthermore, the spherical vacuoles of the alcohol-fixed tissue are of the same relative size and abundance as the globules of the Kopsch-fixed tissue, indicating that the former are the negatives of the latter after solution by alcohol, and contradicting the possible argument that the lipid droplets of the osmic-acid-treated tissue are the product of a precipitation of this acid in this tissue.

The presence of vacuoles in the cytoplasm of these cells must be considered from the standpoint of a possible relation to a trophospongium. In the alcohol-fixed tissue the cytoplasm occasionally appears channeled by a system of irregular canals. The same is true in lesser degree of certain cells in the Kopsch-fixed tissue (fig. 11). Such a system of canals simulates the trophospongium of Holmgren. The appearance here must be interpreted, I believe, as the result of a partial coalescence and distortion of the vacuoles left after either the fixation-solution or a physiologic liquefaction and elimination of the lipid bodies of certain of these cells.

The results of this investigation of the cytology of the pineal body of the young sheep suggest the conclusions that these cells lack a centrosome, that they proliferate amitotically, that they lack both a genuine Golgi apparatus and a trophospongium, and that they are characterized by an abundance of granular mitochondria and a variable number of larger lipid globules. In the younger cells certain mitochondria are bacillary in type; in the later stages hollow-sphere types or ring forms occur. There is no definite line of demarcation, either dimensional or tinctorial, in Kopsch-fixed tissue, separating the mitochondria from the smaller spherical lipid droplets. The two sustain to each other a fairly definite reciprocal numerical relationship. Whether there prevails also a genetic relation remains undetermined. Other than the presence of lipid spherules, there is no indication in these cells of a secretory function.

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PLATE 1

EXPLANATION OF FIGURES

The figures were drawn with the aid of an Abbe camera lucida, a 1-16 Leitz oil-immersion objective, and a no. 10 ocular. The magnification is approximately 1500 diameters. The cells are all of the 'interneuroglia' type, from the pineal body of a four-month-old sheep. The tissue was fixed for four weeks in a 2 per cent solution of osmic acid. The cells selected for illustration represent types of large groups of cells including many minor variations.

1 and 2 Less differentiated cells. The cytoplasm is crowded with mitochondria, predominantly granular, but including bacillary and ring forms.

3 Cell with spheroidal granules on the border-line between mitochondria and larger lipoid globules.

4 Cell with granular and annular mitochondria; and a structure (at lower pole of nucleus) simulating a small Golgi net enveloping a centrosphere. The structure is actually a liquefying and dissolving large lipoid body.

5 Cell with several similar structures, simulating Golgi nets.

6 Cell containing lipoid bodies which grade without sharp line of demarcation from 'mitochondria' into larger lipoid globules.

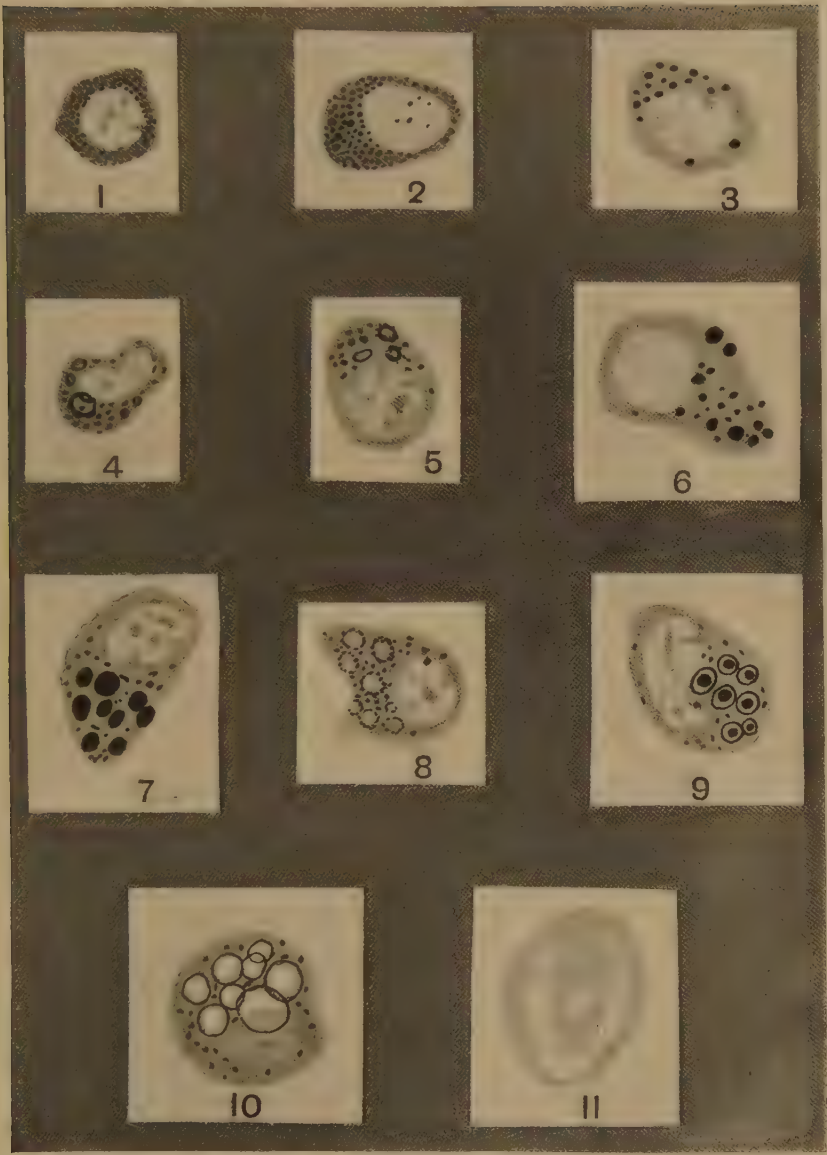
7 Cell with relatively many large lipoid bodies and few mitochondria.

8 Similar cell at later (physiologic?) stage where the large lipoid bodies have become liquefied and have lost their deep brown color, coincidentally effecting an arrangement of the granular mitochondria about their periphery in the form of a granular envelope.

9 Cell in which the lipoid bodies are undergoing solution, giving the appearance transiently of a clear sphere with a thin deeply staining hull and a central granule.

10 Cell at later stage, when the large lipoid bodies have become almost completely liquefied.

11 Cell in which the liquefied lipoid bodies (or their negatives) have coalesced, producing thus a canalicular formation simulating a trophospongium. The section passed outside the location of the nucleus.



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Resumen por el autor, E. V. Cowdry

Células flageladas en la tiroides del perro de mar (*Mustelus canis*).

Las preparaciones de la glándula tiroides del perro de mar teñidas con la modificación del método argéntico de Cajal introducida por Da Fano revelan el hecho de que cada célula está provista de un largo flagelo que se extiende dentro de la substancia coloidal. Puesto que Ferguson y otros han demostrado que los folículos son cavidades cerradas, esta flagelación de las células de la tiroide parece ser una interesante combinación de funciones motriz y secretora sin un valor adaptativo aparente. Puede sin embargo ser de algún interés en relación con el problema del origen de la glándula, indicando tal vez su desarrollo a expensas de un epitelio flagelado semejante al endostilo de las ascidias. Demuestra que la dirección primaria de la secrección es hacia la cavidad del folículo, como ha indicado Bensley, no hacia los vasos periféricos conforme supone Norris.

Translation by José F. Nonidez
Cornell Medical College, New York

FLAGELLATED THYROID CELLS IN THE DOGFISH (MUSTELUS CANIS)

E. V. COWDRY

*The Laboratories of the Rockefeller Institute for Medical Research, New York, and
the Marine Biological Laboratory, Woods Hole, Massachusetts*

ONE PLATE (TWELVE FIGURES)

Preparations of the thyroid gland of the dogfish, made by Da Fano's ('20, p. 157) modification of Cajal's silver method, reveal the fact that each follicular cell is provided with a large flagellum which extends into the colloid substance. The flagella are blackened and show up distinctly in a golden-yellow background (figs. 1, 2, 4, and 5). The reaction is specific and calls to mind Levaditi's silver method for the staining of *Treponema pallidum*. In both cases there is a preliminary fixation in a formalin mixture followed by silver impregnation and reduction. The chief difference lies in the fact that Levaditi's method is designed for smears instead of for sectioned material. My best preparations of flagella have been impregnated for from four to eight days, which is too long for the satisfactory demonstration of the reticular apparatus. Having once studied them by this method, it is not difficult to recognize them in fragments of thyroid fixed in the usual way (formalin-Zenker, formalin-bichromate-sublimate, etc.) and stained with iron hematoxylin (figs. 3, 6 and 9).

Special methods like those of Loeffler and Bowhill, devised for the coloration of flagella in bacterial smears and depending upon the selective mordanting action of tannin, are not helpful. I have been unable to observe the flagella in living thyroid follicles teased out in sea-water, even after the slow addition of iodine, tannin, magenta, fuchsin, and other reagents which usually bring them to light in protozoa; neither have I been able to distinguish them with the aid of the new Litz dark-field microscopic stage apparatus. It is possible that they are masked

by the colloid substance in which they are imbedded. The difficulty of trying to analyze a tissue several cells in thickness in which, when viewed with oblique illumination, there are so many high lights, has prevented me from obtaining decisive results. Isolated thyroid cells give but little information, because, having suffered so much from traumatism, they lose their characteristic shape and cannot be either identified or oriented with certainty.

Using Minchin's ('12, p. 53) criteria of distinction, I have called the filaments 'flagella' rather than 'cilia,' because of their relatively large size, because only one is provided for each cell, and, lastly, on account of their characteristic shape suggestive of free movement in all directions. As seen in the silver and iron-hematoxylin preparations, they present all the morphological characteristics of typical and permanent motor mechanisms. It will be noted that their organization is far more complicated than that of the transitory pseudopodia which Kite described some years ago in erythrocytes.

In silver preparations they sometimes appear to be beaded, as illustrated particularly in the uppermost cell in figure 1; but staining with iron hematoxylin shows that they possess, in reality, smooth and even outlines (figs. 3 and 6). The portion lying in the colloid substance is of even diameter and does not taper noticeably at the end. The fact that its shape varies in different thyroids, as well as in individual follicles of the same gland, seems to indicate different degrees of physiologic activity. Contrast the gentle curves seen in figures 3 and 6 with the typical spirals shown in figure 5. I have been unable to distinguish between the contractile axial filament and its ectoplasmic investment in the free portion of the flagellum, but in specimens fixed in formalin-Zenker and stained with iron hematoxylin the cytoplasmic part is shown with diagrammatic clearness (fig. 3). The flagellum is inserted about the center of the follicular margin of the cell, where, after penetrating through the cell membrane, the ectoplasmic sheath terminates in a small enlargement which stains intensely with iron hematoxylin or blackens with silver, depending upon the technique used. A very slender axial filament continues in the direction of the nucleus and ends in a typical blepharoplast.

The blepharoplasts are intensely blackened with silver, as one would expect from their centrosomal nature. They are also very closely associated, in a topographic sense, with the reticular material (figs. 4 and 7) which separates them from the nuclei. Unfortunately, the silver method which I have been using does not bring both to light simultaneously in the same thyroid cell. The living cells contain a large number of lipid granules which may be supravitaly stained with neutral red. They may also be fixed in Bensley's ('10, p. 192) formalin-bichromate-sublimate mixture and stained with iron hematoxylin. In such preparations it will be seen that they are reduced in number in the immediate vicinity of the blepharoplasts (fig. 6). This reduction in cytoplasmic granulation is also a centrosomal characteristic.

The colloid substance is optically homogeneous, but may be differentially stained by the addition of a little acid fuchsin to the sea-water in which the follicles are being studied. After a few minutes, it becomes slightly granular, but the granules are stationary, exhibiting no currents or eddies which one might expect flagellar action to produce.

We may infer that the flagella are not transitory structures which are thrown out and withdrawn by the follicular cells in different phases of secretory activity, because, as far as I can ascertain without cutting complete serial sections, all the cells of the gland, regardless of their physiologic condition, are provided with them. They are as well developed in swollen cuboidal cells as they are in flattened cells, though the latter are generally supposed to be less active functionally. This may be seen by comparing figures 1 and 2. Cells whose nuclei are displaced toward the follicular lumen through the heaping up of colloid substance in the cytoplasm near the peripheral blood vessels possess typical flagella which are in no way altered (fig. 8). In these 'colloid' cells the lipoidal granules are also pushed toward the lumen (fig. 9), but the reticular material retains its usual position (fig. 7). Neither do I believe that the flagella are restricted to certain individuals of the species, because I have repeatedly confirmed their existence in adult dogfish captured

and examined as follows: June 18th, 20th, 23rd, 26th, 29th, and August 23rd.

I have examined several other elasmobranchs to determine whether flagellation is a phenomenon of wide occurrence which has been overlooked because investigators have not been in search of it. Ordinary preparations do not reveal it unless they are examined with special care. Energetic coagulants cause a shrinkage of the colloid substance which tears the flagella away from the cells, leaving them with a perfectly smooth border. One dusky shark (*Carcharinus obscurus*) and one sting ray (*Dasyatis centroura*) show no trace of flagellation, but flagella are undoubtedly present in some at least of the thyroid follicles of one summer skate (*Raia erinacea*). They are illustrated in figures 10, 11, and 12. In this single specimen I have been unable to detect typical blepharoplasts, though I strongly suspect their occurrence. Each cell seems to possess a single flagellum which, as in the dogfish, may be wavy or spiral in shape, suggestive of internal tension; but the thyroid is so aberrant in its general structure that I hesitate to draw any very definite conclusions on the basis of insufficient material which I have not had an opportunity to re-examine. The follicular lumina, in addition to being invaded by large numbers of leucocytes, as noted by Ferguson ('11, p. 206) in *Carcharias*, contain concretions of great variety as well as large isometric crystals which can be readily seen unstained in living follicles in sea-water. Moreover, the epithelium of individual follicles occasionally varies in height from low cuboidal to tall columnar cells. Some of the follicles communicate and branch. It is possible that further study of the dusky shark and the sting ray will likewise reveal flagellation.

The uniform flagellation of the entire thyroid in the dogfish is quite different from the ciliated cavities which are occasionally described in treatises on the pathologic anatomy of the human thyroid gland. The ciliated cavities are directly derived from the pharyngeal epithelium, do not contain typical colloid substance, and are not lined by true thyroid cells. Ferguson ('11, p. 191) has shown that in the dogfish the thyroid follicles are isolated structures with closed cavities which are not in any way

connected with the pharynx by a duct system. It is a simple matter to verify this independence by teasing a small fragment of tissue in a dilute solution of neutral red in sea-water which stains the lipoid content of the follicular cells intensely and delimits them sharply.

Norris ('18, p. 216) has studied the development of the thyroid in a closely related form, *Squalus acanthias*, and finds that the thyroid, as in other vertebrates, is at first a solid outgrowth of the pharyngeal epithelium in which the follicular cavities develop secondarily. What, we ask, is the stimulus which calls forth the formation of a flagellum in each of these follicular cells? It is almost inconceivable that such an elaborate mechanism should not serve some useful purpose. Although I have not actually observed movement of the flagella, they have all the distinctive characters of a typical motor apparatus such as one meets with in the protozoa or in hydra.

A special mixer to stir up the follicular contents is perhaps less needed in the dogfish than in mammals, because the thyroid lies in the median line on the coracohyoideus muscle immediately posterior to the mandible and is covered by the constrictor pharyngis and coracomandibularis muscles, so that pressure is brought to bear upon it with each movement of the jaws. There are some unique features in the general physiology of the dogfish; but I am unable to find anything approaching a clue. In the absence of direct information from extirpation experiments, about all we can say is that, unlike the mammalian thyroid, the thyroid of the dogfish plays no part in the development of a bony skeleton, because no such skeleton is present in elasmobranchs. It has been suggested by F. D. Thompson that the thyroids of elasmobranchs may have to discharge some of the functions of the parathyroid glands which are not recognized below the amphibia. The absence of a physiologic reserve of glycogen in either the liver or muscles of the dogfish¹ suggests a marked difference in metabolism which may in some way be reflected in the thyroid gland. There is also a considerable amount of

¹ Personal communication from Dr. H. C. Bradley.

urea in the blood. But it seems incomprehensible that these factors should have any direct bearing on flagella formation.

While I do not venture to suggest that these thyroid cells have developed flagella merely because they acquired the habit years ago, a brief consideration of the ancestry of the gland may be helpful. According to the most widely accepted theory, originally advanced by W. Müller, the thyroid is considered to have developed in the course of evolution from some structure like the endostyle of ascidians. This endostyle is an epithelial groove in the ventral wall of the pharynx along which food is passed to the stomach. It is lined with several longitudinal rows of cells possessing stout cilia (or flagella) and also by mucous cells which become more numerous near the stomach. The gland cells produce a thick adhesive secretion in which minute microorganisms and other food particles are entangled and become rolled up by the action of the cilia into a rounded bolus of suitable size for digestion. The evidence for homology with the mammalian thyroid is not very direct. It consists of close correspondence in position and of strong collateral evidence that ascidian-like forms are in the line of our ancestry. It is, in our opinion, a little unsafe to postulate a close genetic relation between the mucous secretion of the endostyle and the mammalian colloid, because they are so different chemically and apparently serve different functions, but the flagellation, which I have found in the dogfish, may be of some phylogenetic significance.

It is interesting to note in this connection that Camp ('16, p. 406) has studied the development of the suprapericardial body in *Squalus acanthias* and finds that it "represents the ventral extremity of a rudimentary seventh gill pouch" and that it consists in part of closed follicles containing goblet cells which secrete mucus. While it has not been definitely shown that the suprapericardial bodies find their homologues in the lateral thyroids of mammals, their striking resemblance to thyroid tissue and the presence in them of mucous cells may have some bearing upon our problem.

In the same way that the flagellated thyroid cells of the dogfish may be distantly related to the ciliated (or flagellated?)

cells of the endostyle, so the mucous cells of the suprapericardial body in *Squalus acanthias* which develop from the pharyngeal wall farther back and nearer the stomach, may be related to the mucous cells of the endostyle which are said to be more abundant in its posterior part. It is possible, therefore, that we have represented in the thyroid and pericardial bodies of elasmobranchs all the known components of the endostyle of tunicates. Unfortunately, our actual knowledge of the minute structure of the endostyle is very scanty, because investigators seem to have contented themselves with the descriptions published by Fol, Müller and Dohrn, written about forty years ago without much regard for cytologic detail.

We may at least conclude that the presence of flagella extending from the cells into the follicular cavity indicates that this cavity originally communicated with the pharynx; in other words, that the flagella constitute definite clues to the primary polarity of the cells.

This homology of the follicular cavity with the lumen of the ancestral gland, whatever it may have been, is difficult to reconcile with Norris's ('18, p. 214) hypothesis. He finds that the thyroid exhibits intraglandular cavities which are quite independent of the original thyroid pouch at approximately the same stage in the development of *Squalus acanthias*, birds, and mammals. While admitting that any attempt to interpret "their immediate or general biologic significance, although interesting, can at best be only speculative," he suggests that they "appear in response to a tendency to reproduce the ancient lumen or duct of the ancestral gland." As development progresses, they open on the surface of the gland and become invaded by mesenchyme and blood vessels. The epithelial cells in their walls become disposed in two layers, between which the definitive follicular cavities develop so that in the adult the primary polarity of the cells bordering the follicles is (according to him) directed toward the peripheral blood vessels (which occupy his intraglandular spaces), and Bensley's conclusion "that the thyroid cell represents a true reversal of polarity" is

quite unnecessary. I am unwilling to accept this suggestion for two reasons:

In the first place, all digestive glands which develop through an evagination of the wall of the primitive alimentary tube seem to maintain their epithelial lining intact. We are asked to believe, in the case of the thyroid, that this epithelial investment breaks down completely and that blood vessels, lymphatics, nerves, and connective tissue grow into the intraglandular spaces, or primitive lumina—biologically speaking, that they migrate outside of the organism, for the duct was, we suppose, originally in direct communication with the outside environment. This is a process, to the best of my knowledge, without counterpart in the development of other glands.

In the second place, Norris's claim (p. 215) that these spaces are quite independent of the follicular cavities in *Squalus acanthias*, as well as in all representative vertebrates which he has studied, would point to the conclusion that they are also independent in the dogfish, which is a closely related form. Since we know by their flagellation that the follicular lumina must represent the ancestral duct, it follows that we must seek some other explanation for the intraglandular spaces of Norris.

We may, therefore, accept Bensley's conclusion that, when in the mammalian thyroid secretion passes directly into the blood vessels, instead of into the follicular lumen (ancestral duct), we are actually dealing with a true anatomic and physiologic reversal in polarity.

SUMMARY

The development of flagella in the thyroid gland of the dogfish is an interesting combination of motor and secretory functions without apparent adaptive value. It may, however, have some bearing upon the problem of the ancestry of the gland indicating perhaps its development from a flagellated epithelium like the endostyle of ascidians. It does show that the primary direction of secretion is toward the follicular lumen, as claimed by Bensley; not toward the peripheral blood vessels, as suggested by Norris.

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PLATE 1

EXPLANATION OF FIGURES

All the figures have been drawn with a Zeiss apochromatic 2-mm. objective, compensating ocular, no. 8 and camera lucida and are reproduced without reduction. Figures 1 to 9 are of the thyroid of the dogfish (*Mustelus canis*) and figures 10 to 12 of the skate (*Raja erinacea*).

1 Thyroid cells with large flagella blackened by the silver method. At the base of each flagellum a typical blepharoplast may be seen.

2 The same showing that the size of the flagellum is not decreased when the thyroid cells become flattened.

3 Thyroid cells fixed in formalin-Zenker and stained with iron hematoxylin. The lipoid granules which are illustrated in figures 6 and 9 have been dissolved out so that the axial filaments of two of the flagella can easily be distinguished passing into the blepharoplasts.

4 Thyroid cells prepared by the silver method with the reticular material impregnated and the flagella slightly darkened. Although the blepharoplasts cannot be identified, the axial filaments probably terminate in close association with the reticular material.

5 Thyroid cells treated by the same method showing wave-like and corkscrew-shaped flagella. The flagella of different animals and sometimes of neighboring follicles in the same animal often show great variations in shape suggestive of different degrees of physiological activity.

6 Thyroid cells fixed in Bensley's formalin-bichromate sublimate mixture and stained with iron hematoxylin which preserves both the lipoid granules and flagella. The granules are less abundant at the points of insertion of the flagella.

7 Group of cells prepared by the silver method, showing the gradual accumulation of colloid material in the zone of cytoplasm bordering the peripheral blood vessels with displacement of the nucleus toward the lumen. Since the position of the reticular material is unaltered, we are not dealing with a true reversal in polarity as described elsewhere (Cowdry, 1922) in the thyroid of the guinea-pig.

8 Thyroid cells prepared by the same method, but treated five days longer with silver which has blackened the flagella. In the colloid cell it will be seen that the flagellar apparatus is in no way modified, in spite of the fact that a large amount of colloid material has collected in the peripheral cytoplasm and the nucleus has been displaced towards the lumen.

9 Thyroid cells from the same tissue as figure 6, showing that the lipoid granules remain unmodified in colloid cells. The flagella are unstained. A few rod-like mitochondria may be made out in the peripheral cytoplasm.

10 Thyroid cells of the skate prepared by the same silver nitrate method and bearing slightly undulating and spiral flagella extending into the colloid substance.

11 Cells from the same specimen each provided with a single flagellum. The blepharoplasts cannot be seen.

12 Group of flagellated, columnar cells also from the same specimen. Since the cells are so closely packed together and are not very distinctly outlined, it has been difficult to associate them with their respective flagella.



Resumen por el autor, Edmond Souchon

Un resumen de la conservación de las disecciones anatómicas con color permanente de los músculos, vasos y órganos.

El autor ha usado durante algún tiempo como solución permanente una solución compuesta de cloruro cálcico y formol, pero nuevos experimentos han demostrado que la sal cálcica no es necesaria. El agente activo es el formol. Una solución de 5 onzas de formol en un galón de agua clara conserva los tejidos e impide el enturbiamiento del líquido. El formol no decolora las disecciones preparadas por los métodos Souchon. La evolución de dichos métodos se basa en los siguientes hechos: Primero, que las disecciones de los músculos sumergidas en una solución de glicerina durante algunos días y después abandonadas al aire libre se oscurecerán mucho al cabo de cierto tiempo. Segundo, que el formol actúa como decolorante de los tejidos coloreados normalmente. Tercero, que las arterias y venas pintadas toleran la acción de cualquier solución ordinaria empleada para la conservación de los tejidos.

Translation by José F. Nonidez
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PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS, AND ORGANS

A SUPPLEMENTARY NOTE

EDMOND SOUCHON

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The results of the experimental work on the preservation of anatomic dissections have been published in several articles in *The Anatomical Record* of Philadelphia and elsewhere.

During the evolution of this work it has undergone a number of changes, alterations, substitutions, and additions, etc.

I here publish a supplementary note presenting the final status of the methods.

With regard to the curing method, at the end of three days the preparation is taken out of the glycerin and exposed to the air in a room until the muscles become of a marked brown color. The longer the exposure to the air, the darker the brown. The darker the brown, the darker will be the final color of the muscles in the solution. The shade of the final color can be graded in this way. This curing method has superseded the chemical method.

It is then placed in a permanent solution. For a time I used a solution composed of calcium chloride and formol, but further experimentation has shown that the calcium is not necessary. It is the formol that is the active agent.

A solution of 5 ounces of formol to 1 gallon of clear water will preserve and prevent cloudiness. The formol must be as clear as water. If greenish, it will discolor the solution. If greenish, it should be filtered through bone-black before using to make it clear. Before using the solution, filter it anyhow through bone-black and paper to obtain filtered clearness.

It may be that 3 ounces of formol may do. Experimentation will determine that. The formol will not bleach the dissections prepared by the Souchon method.

THE EVOLUTION OF THE SOUCHON METHOD FOR THE PRESERVATION OF ANATOMIC DISSECTIONS¹

I have always been very much interested in the various phases through which discoveries and inventions went before reaching the goal. For this reason I am giving here the various stages of the Souchon methods.

The two methods rest on three facts, none of which is new separately, but is new in the application or combination. They are: First, that dissections of muscles immersed in solution of glycerin for a few days and then removed and left in the open air or enclosed in a receptacle will become very black in coming time. Second, that formol was a bleacher of colored tissues. Third, that painted arteries and veins would stand the action of any ordinary solution used to preserve the tissues. Although I knew all these facts years ago, it took me ten years of work and experiment to bring together the combinations that brought about the results.

When I found out that glycerin would blacken the muscles, I tried several chemicals to reduce the blackness, and it is only after several years of experimentation that I struck formol, which did the work.

I had used many years ago solutions of gelatin to paint pastel drawings before trying a thin coat of damar varnish, but it was only after two or three years of unsuccessful experiments at painting muscles that I bethought myself of first applying a coat of gelatin to the muscles. That gave success.

The elimination of the calcium chloride from the calcium formol solution is due to the fact that I always follow the rule of Pasteur, which consists, when a work is completed, to go over it critically, changing, etc., with a view of simplifying it.

¹ At the New Orleans Meeting of the American Medical Association in 1920, the Committee awarded to Dr. Edmond Souchon the Gold Medal for the best scientific exhibit of his anatomic dissections. The dissections were made according to the Souchon methods.

The only real new discovery was that solutions of 5 per cent formol would not in time bleach the dissections prepared by the Souchon method. They will bleach dissections prepared by other methods.

Resumen por el autor, Wesley M. Baldwin

La producción artificial de siringomiocèle en el renacuajo por medio de los rayos X.

El autor ha sometido los huevos de la rana toto, rana verde, rana leopardo y sapo común a la acción de los rayos X emanados de un tubo especial de aire frío de Coolidge. Veinticinco de los huevos de la rana toro en una serie de 100 presentan en secciones seriadas una verdadera condición de siringomiocèle. Los defectos notados en los cortes comprenden un desarrollo anormal del corazón, vasos sanguíneos y pericardio dilatados, una circulación estática y una marcada dilatación del neurocele en los niveles segmentarios que indican la zona de unión del cuerpo con la cola.

La dilatación del neurocele está acompañada por un pronunciado adelgazamiento de la pared dorsal del tubo neural, por la posición lateral de los miotomos y por la ausencia de la fusión de las mitades del arco neural.

Translation by José F. Nonidez
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THE ARTIFICIAL PRODUCTION OF SYRINGOMYELO- CELE IN THE TADPOLE BY MEANS OF X-RAYS

W. M. BALDWIN

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At the time of the consideration of "The artificial production of monsters demonstrating the condition of spina bifida as the result of the action of ultra-violet light rays," as published in the *Anatomical Record*, vol. 9, no. 9, May, 1915, the several differences in anatomic structure, differentiating this condition from that group of the same name appearing occasionally in the human embryo, were appreciated. Subsequently, an effort was made to approximate more closely the human condition through the utilization of the same physical agent, but with a variation in the method of application.

It has been ascertained since the publication of the paper on "The artificial production of monsters conforming to a definite type by means of x-rays," *Anatomical Record*, vol. 17, no. 3, November, 1919, that x-ray energy was fully as dependent a physical agent in the production of spina bifida as ultra-violet light ray energy. The mechanics of production appeared to be identical with that reported previously. Certainly, the end-product differed in no apparent histologic detail from that produced by the light rays. As was the case in the instance of the latter, the causative factors were resolvable into a single inhibitive force preventing the medianward migration and fusion of the hemineural tubes.

On the other hand, were the morphologic features the only details to be considered, one might assume, in making a comparison between the spina bifida of human monsters and the spina bifida resulting from experimental methods, that the former condition, in contrast to the tadpole, reflected a com-

paratively complex, causative agency. Manifestly to a single inhibitive force could be referred neither the thinning of both the epidermis and of the sublying mesenchymal tissue, the separation and defective development of the myotome halves, nor the bulging of the cord through a dilatation of its canal or a protrusion of the meninges. It might be assumed, however, that the interposition of an inhibitive force between the neural-arch halves, thereby inducing a separation and lack of coalescence of them, together with a concomitant cleavage of the overlying myotome halves, might permit the cord and its meninges, by virtue of this resultant median cleft, to approach more or less intimately into contact with the overlying epidermis. There would still be lacking under these conditions, however, an explanation of the myelocoelous condition and of the bulging of the meninges. It would appear at first consideration that these latter factors were rather the resultant of a generalized force acting upon the vascular system as a whole including the lymphatic system, with possibly a sharply circumscribed defect of the neural tube.

Incidentally, such defects have been noted previously and reported in the earlier papers as accompanying marked morphologic defects of the heart. Such have been characterized by dilatation of the neurocele and thinning of the dorsal parietes of the neural tube. The superabundant cerebrospinal fluid, together with the bulging membranes of the cord, and the dilated neurocele seemed altogether to point directly to a generalized defective condition of the vascular system, most probably localized in part, at least, in the heart, which had induced a stasis of circulation.

In several of the specimens reported in the paper of November, 1919, it was noted that the expanded encephalocele with its thinned dorsal parietes occasionally featured the neurocele as well and, singularly enough, at a most usual level at the junction of the trunk and tail. The expanded pericardium, together with the dilated lymph spaces under the ectoderm, furnished contributory evidence upon the essential causative factor, the imperfect development of the heart. If these inferences were correctly

drawn, therefore, the agencies underlying the condition of human spina bifida must be referable to, at least, two separate and independent group factors—one responsible for the separation of the neural arch-halves and of the myomeres, and the other for the dilatation of the neurocele and the consequent thinning of its dorsal wall together with the bulging of the meninges.

The author has thus far succeeded in producing one type of spina bifida, resembling, in every histologic detail, syringomyelocele in the human. The experimental production of this condition cannot be controlled at present, however, with the same degree of certainty as was true of spina bifida of the tadpole. The developmental agencies which underlie the defect have been recognized as the two which are enumerated above. The causative physical agent employed was x-ray energy.

The eggs used for the experiment were those of the bullfrog. These were obtained and rayed during the one- and two-cell stages. A general exposure of the egg for two minutes was given at a distance of 4.8 cm. from the center of the target. The apparatus consisted of a special Coolidge air-cooled x-ray tube, such as was described in the *Anatomical Record* of November, 1919. Upwards of 100 eggs were used in the study, twenty-five of which showed, upon careful study of the serial sections, the condition of syringomyelocele. These were the optimum results with 100 eggs. Unfortunately, it has not been possible to increase the percentage of production of this anomalous condition through repeated experimentation. With other types of eggs, such as the green frog, leopard frog, and common toad, the percentage of production fell much lower.

The most conspicuous external feature of these tadpoles was a dwarfed appearance together with a relatively prominent and bulging trunk. The total length of the body was about one-fourth less than that of the controls, while the tail accentuated this shortening over the controls by as much as one-half. The head parts, though reduced in size, were well formed. This was especially true of the brain. Their swimming reactions were sluggish, resembling in this respect the above-mentioned tadpoles, but all movements were carried out normally.

A study of the histologic sections demonstrated features which so far as related to the cardiovascular and nervous systems were identical to those described in the previous paper. They require no added description here. At the junction of the trunk and tail, however, the anatomic arrangement merits special attention. At this level, extending at the most a distance of two or three segments, the neurocele was expanded. The major part of the dilatation affected chiefly its dorsal parietes, thereby producing an attenuated wall. The myotomes in the affected segments were normally formed, but lay somewhat farther laterally than is usually the case. This dorsal expansion of the neurocele had the effect of bringing its dorsal wall into close contact with the ectoderm, the ventral wall being in approximately its usual position relative to the notocord. A few mesenchymal cells occupied the narrow interval between the neural tube and the ectoderm. To all histologic appearances, the ectoderm was normal.

It was necessary to trace the several developmental steps in the production of this condition in order to ascertain by which of the two possible methods this anomalous condition had been brought about. The approximation of the neural tube to the ectoderm was not the resultant of a delayed separation of the neuroblasts of the neural groove from the latter. The early developmental stages of the neural ridges and groove, comprising the separation of the latter from the ectoderm, progressed in a perfectly orderly and normal fashion. This stage was followed in these defective embryos, as well as in the controls, by the interposition of a mesenchymal layer between the ectoderm and the neural tube. During those later stages, however, when the defective development of the heart was made manifest through dilated vessels, pericardium, and a static circulation, then the dilation of the neurocele at the segmental level mentioned became pronounced. This expansion affected the dorsal wall of the neural tube more markedly. Apparently, as a direct result of pressure thereby applied, the mesenchymal elements were forced out of the interval between the tube and the ectoderm. At the same time, the neural arch-halves were restricted laterally, as was true of the myotomes as well.

Extended experimentation with waves of varying length has demonstrated to the mind of the author the outstanding fact that if such a condition as specific cytologic susceptibility to x-ray activity exists, the greatest single factor to which that phenomenon may be referred is the relative mitotic activity of the various types of cells. That is to say, where a sufficient amount of x-ray energy of the usual wave-length is employed, it is possible to influence directly all types of cells of embryonic tissues. The single determining factors are, apparently, the quantity of energy utilized plus the rate of division of the cells. As development progresses, the varying degrees of absorption capacity are in direct proportion to the rapidity of mitotic succession in those cells. This accounts in part, at least, for the observed greater absorption capacity of young and growing neuroblasts, in contrast to the relative lack of that property in adult cells of the same type. That there has been in the experiments given a direct inhibitive action upon the neuroblasts is evidenced through both staining and morphologic features. But the physiochemical mechanism underlying the increased absorption by cells of a single localized area in the caudal extremity of the trunk does not appear through a study of the experimental data. The defects noted in the cardiovascular system constitute apparently an essential element as well in the production of this defect. In no specimen yet studied has the condition of syringomyelocoele been produced without an additional manifest cardiovascular defect as well. The question naturally arising, as to whether the defect in both systems, the neural and the cardiovascular, represent a single phase of cytologic reaction to x-ray activity, still awaits demonstrative evidence.

Resumen por el autor, Otto F. Kampmeier

Un caso notable de asimetría de la región tiroídea asociado con la presencia de un quiste branquial.

En la región cervical anterior de un varón negro de veinte años, fallecido a consecuencia de tuberculosis miliar, ha encontrado el autor las siguientes anormalidades: 1. Ausencia completa del lóbulo izquierdo e istmo de la tiroides, con una ausencia correspondiente de las arterias tiroideas superior e inferior; el lóbulo derecho presentaba tamaño normal. 2 Ausencia completa del músculo esterno-tiroideo derecho; el músculo correspondiente del lado izquierdo presentaba tamaño mayor que el normal. 3. Presencia de una sola paratiroides en la región tiroidea inmediata. El autor no halló otras paratiroides. 4. Presencia de una tiroides accesoria (de 1.5 cm. de diámetro) situada inmediatamente debajo del lóbulo derecho de la tiroides; la irrigación sanguínea y el drenaje de la glándula demuestran que no se trata de un lóbulo izquierdo de la tiroides desplazado. 5. Presencia de un quiste cervical o branquial rodeado de un epitelio (de 2.5 cm. de longitud por 1.2 cm. de anchura) el cual, situado al nivel del cartílago tiroides, estaba aplicado al lado izquierdo de la farínge extendiéndose hacia arriba hasta el asta mayor del hioides. El carácter de las asimetrías y anomalías mencionadas indica que se originaron en fases tempranas de la vida embrionaria, durante la época en que los derivados glandulares de la farínge y de la musculatura branquial y cervical comenzaron a formarse y diferenciarse. Del mismo modo no puede dudarse que en la producción de estas diversas anomalías estas estaban interrelacionadas de un modo definido durante su formación y que pueden referirse a un factor nocivo común.

Translation by José F. Nonidez
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A STRIKING CASE OF ASYMMETRY IN THE THYROID REGION ASSOCIATED WITH THE OCCUR- RENCE OF A BRANCHIAL CYST

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ONE FIGURE

The following observations were made on a negro male, twenty years old, who died at the Cook County Hospital of miliary tuberculosis, and whose body was dissected during the past school year.

When the skin and subcutaneous tissues were being reflected from the region of the anterior triangle of the neck, nothing unusual was noted. Both the omohyoid and the sternohyoid muscles were broad and well developed and were situated in their normal position. But when these muscles were reflected a striking asymmetry of the underlying structures became apparent. The left lobe of the thyroid and the isthmus were entirely absent,¹ and on the right side there was no trace of a sternothyroid muscle, while the corresponding muscle on the left side was much larger than usual. Later, when a search was made for the parathyroids, only one was found in the immediate thyroid region. Still later a cyst was discovered behind the larynx to the left of the pharynx.

The right or existing lobe of the thyroid was of normal shape and size, and occupied the usual position as indicated in the figure (*thyr.*). Its surface showed distinct lobulations, it was firm, and in section showed no abnormal characteristics.

Situated at the inferior margin of the single thyroid lobe occurred a rounded mass of tissue (fig. 1, *thyr. acc.*) approxi-

¹ The writer thanks his students Messrs. Leitch, Lenzen, Goebel and Brough for calling his attention to the absence of the left lobe of the thyroid and for their carefulness in following his instructions in the subsequent dissection.



Fig. 1 The figure shows the skin and subcutaneous tissue removed from the region of the anterior triangle of the neck and the sternocleidomastoid, omohyoid, and sternohyoid muscles cut to expose the underlying structures, such as the trachea, thyroid, the carotids, jugulars, thyrocervical trunk and branches, etc. Approximately one-half natural size. *a. thy. sup.*, arteria thyroidea superior; *a. thy. inf.*, arteria thyroidea inferior; *v. thy. sup.*, vena thyroidea superior, this branch is absent on the left side; *v. thy. inf.*, vena thyroidea inferior (on account of its relatively high position, it might be called vena thyroidea media; the corresponding vein is absent on the right side); *v. thy. ima*, vena thyroidea ima; *m. stithyr.*, musculus sternothyroideus (absent on the right side); *thy.*, right thyroid lobe (left lobe absent); *thy. acc.*, accessory thyroid; *parathyr.*, cross-barred area indicates position of the only parathyroid found (beneath sternothyroid muscle); *cyst*, cross-barred oval area indicates position of the branchial cyst between larynx and pharynx.

mately the size of a marble, though somewhat flattened, which on microscopic examination of a section was found to be composed of thyroid follicles. At first it was believed that this small thyroid nodule represented a much reduced and displaced left thyroid lobe, but study of its vascular supply and drainage led one to a different conclusion. The right superior and inferior thyroid arteries (*a. thyr. sup. et inf.*), which in their origin from the carotid and thyrocervical trunk, respectively, were normal, not only supplied the right thyroid lobe, but after ramifying through its substance sent small terminal branches to the inferior nodule. Moreover, the inferior thyroid vein (*v. thyr. inf.*) of the left side drained only the thyroid lobe and not the nodule, while the thyroid ima vein (*v. thyr. ima*) drained both. The right inferior vein was absent. These observations would seem to indicate that the small separate thyroid nodule, instead of being a reduced and displaced left lobe, represented an accessory thyroid, which condition occurs relatively frequently, as is well known, and which apparently results during the early development of the gland, when the proliferating cords of cells break up into the anlagen of the follicles and the ingrowing connective tissue divides the gland into more or less definite lobules. It is assumed, however, that in this case the formation of this accessory thyroid was related in some way to the causes which determined the asymmetrical development of the neighboring structures.

On the other side, no trace of a left superior thyroid artery was found, and what was believed to represent the vestige of a left inferior thyroid artery was so minute that it was impossible to follow it out to its termination. It was lost in the connective tissue lateral to the trachea and beneath the sternothyroid muscle (*m. stthyr.*).

The asymmetry of the sternothyroid muscle, normally paired, was very interesting. No vestige of this muscle was found on the right side; not even an aponeurotic band or strand was found stretching across the anterior or ventral face of the thyroid. On the left side, on the contrary, the sternothyroid muscle (*m. stthyr.*) was well developed and stretched between its normal

points of attachment on the manubrium sterni and the thyroid cartilage. It was thicker than usual and with the underlying connective tissue filled out the space normally occupied by the lobe of the thyroid. The thyrohyoid muscles were present on both sides.

In searching for the parathyroids, all the small nodules, six in number, in the immediate vicinity of the thyroid were removed and sectioned, after their positions had been plotted on a drawing. Only one of these structures, however, was found to be composed of parathyroid tissue; the remaining ones were small lymph nodes. This single parathyroid had the shape and size of a small pea, and was situated in the connective tissue immediately dorsolateral to the trachea at the level of the cricoid cartilage. Its position has been projected on the overlying sternothyroid in the figure (*parathyr.*). Other parathyroids, if present, must have occupied positions much farther removed from the thyroid than is normal.

The cyst mentioned above represented a flattened tough sac, rectangular in outline and approximately 2.5 cm. long, 1.2 cm. wide, and 0.6 cm. thick (dorsoventral diameter). It was located at the level of the thyroid cartilage against the left side of the pharynx, and its upper end extended as far (fig. 1, *cyst*) as the larger horn of the hyoid bone. The walls of the sac, although tough and resistant, were relatively thin, semitransparent or translucent, and showed a yellowish fluid content within its cavity. This sac was attached by two tough connective-tissue strands, an inferior one, passing downward a short distance and disappearing in the connective tissue between larynx and pharynx, and a superior one, 3 or 4 cm. long, joined to the fascia covering the pharyngeal musculature.

After the location, attachment, and topographical relations of the cyst had been noted, it was removed from the cadaver, opened, sectioned, and stained. Microscopic examination showed an epithelial lining, composed chiefly of a stratified epithelium of five or six layers of cuboidal or polygonal cells. In certain areas, however, the cells formed only a single or double layer. Outside this epithelium was a layer of fibrous connective tissue, not very wide, but rather compact and dense.

Considering the existence of several possibilities of origin, it was of course impossible to determine the exact embryological derivation of the cyst. It might represent the persistence of any one of the last two or three pharyngeal or branchial pouches of the embryo, or it might have developed from a persisting postbranchial body, a structure of problematical significance derived from the vestigeal fifth branchial pouch and normally disappearing in the lateral lobe of the thyroid during the latter's development and expansion. It can hardly be assumed that the cyst may have developed from a part of the original thyroid anlage which had become isolated from the main mass during its migration from its initial site beneath the tongue to its definitive position, for the location of the cyst dorsal or posterior to the larynx would not support such an assumption.

The character of the asymmetries described above, especially the absence of any vestiges of the left thyroid lobe and the right sternothyroid muscle, would suggest that these abnormalities began early in embryonic life, at the time when the glandular derivatives of the pharynx and the branchial and cervical musculature began to form and differentiate. Which factor disturbed the normal developmental process in this region is impossible to say at the present time. But there seems to be no doubt that the production of these different abnormalities were definitely interrelated in their formation and can be referred to a common disturbing factor.

In perusing the literature on the abnormalities and malformations in the thyroid region, the author found only two or three cases in which the absence of one of the lateral lobes of the thyroid was reported, and only two cases in which the sternothyroid muscles were completely absent. In 1852, Handfield Jones classifies among other anomalies of the thyroid, exhibited in the museum of Guy's Hospital, London, a preparation in which only one lobe of the thyroid was developed, associated with a little glandular body situated below the middle of the thyroid cartilage. In 1862, Luschka, in his text-book of anatomy, reports the case of a complete absence of one lobe of the thyroid in a new-born. In 1883, Gow observed the absence of the left thyroid lobe in

an old woman. He finds the superior and inferior thyroid arteries of the corresponding side present, but very minute. He states that the right thyroid lobe was not enlarged, an observation also made by the present writer, who did not notice any compensatory hypertrophy of the remaining lobe. A condition apparently as rare as the absence of one of the thyroid lobes is the complete absence of the sternothyroid muscles, which has been noted by Otto (1816, 1824) and Chudzinski ('94). In these cases, the muscle was found absent on both sides.

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Resumen por los autores, Raymond L. Barney y Barry J. Anson

Abundancia estacional del pez destruidor de mosquitos, *Gambusia affinis*, especialmente en relación con su fecundidad.

El estudio de cortes de los órganos reproductores de las hembras grávidas y no grávidas de *Gambusia affinis* ha demostrado la existencia de un tejido que envuelve exteriormente al ovario y la de un "conducto genital" funcional que conduce las larvas al seno urogenital después que han abandonado los folículos ováricos. Por medio de un exámen microscópico de los ovarios de unos 800 individuos capturados durante todos los meses del año los autores han obtenido pruebas que indican claramente que en Mound, Louisiana, todos los huevos de una *Gambusia* hembra de 3.3 cm. o mayor longitud, y tal vez los de las hembras de menor tamaño, que aparecen más tarde durante el mismo año en forma de larvas, son fecundados simultáneamente.

La producción de huevos en esta especie es un fenómeno cíclico, setando regulada la cuota anual de peces jóvenes por el tamaño y potencias metabólicas de la hembra, y no solamente por la temperatura del agua favorable para las actividades de la cría. Existe un periodo de descanso en el ovario que dura desde el nacimiento de las larvas a fines de Septiembre y en Octubre hasta la época de la fecundación, en la primavera siguiente. La relación del tamaño de la hembra y la temperatura del agua con la fecundidad es indicada por los autores. Se ha observado una correlación entre la rapidez estacional de la liberación de las larvas de *Gambusia*, la abundancia estacional y la "frecuencia de los jóvenes" de la especie en las condiciones naturales.

Translation by José F. Nonidez
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THE SEASONAL ABUNDANCE OF THE MOSQUITO- DESTROYING TOP-MINNOW, *GAMBUSIA AFFINIS*, ESPECIALLY IN RELATION TO FECUNDITY

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THREE FIGURES AND TWO PLATES (SIX FIGURES)

In previous papers the authors ^{1, 2} have shown the changing seasonal abundance of *Gambusia* and how varying frequency of potent males, oxygen content of water, plant associations, and other general environmental features affect the natural history of this viviparous top-minnow. The value of such knowledge in its relation to a practical mosquito-control campaign has been pointed out in this connection. As a supplement to these publications, the present paper is offered as a further consideration of the natural history of *Gambusia* with special reference to fecundity in its general bearing on seasonal abundance of this species in nature. This study corroborates and is correlated to some previous conclusions concerning changing seasonal abundance and gives certain added information concerning productiveness in this minnow.

The fish examined and discussed herewith were collected at Mound,* Louisiana, during the years 1916, 1917, 1918, and 1921. Examinations of the fish were made by the junior author and Mr. H. Walton Clark. Approximately 800 female *Gambusia*, collected from many points along bayous, borrow-pits, wood lakes, and pools, representing several different environ-

* The authors were employed as representatives of the U. S. Bureau of Fisheries in a cooperative study of fish control of mosquitos with representatives of the U. S. Bureau of Entomology. The observations were made from the latter's field laboratory at Mound. Special thanks are due Dr. W. V. King for suggestions and for the collection of certain of the *Gambusia* discussed in this paper. We are indebted to Dr. R. E. Coker for criticism of the manuscript and for suggestions.

mental associations discussed in previous papers, were examined. Counts were made of all embryos.

The anatomy of the female reproductive organs and the embryology of *Gambusia* are so unusual that a résumé of the facts bearing on these subjects seems warranted, in view of their relationship to further discussion in this paper. We take the liberty to quote in this connection the careful observations of Ryder⁷ (pp. 144 and 145):

The ovary is a simple, unpaired organ, the greater part of which lies on the right side of the body-cavity below the air-bladder, and serves to fill up the greater part of the inferior moiety of the former when developed to maturity with its follicles gravid with embryos. The ova, when full grown, are each enveloped in a sack or follicle supplied with blood from a median vascular trunk, which divides and subdivides as it traverses the ovary lengthwise in a manner similar to that of the stem to which grapes in the bunch are attached. . . .

Every fully-grown ovum, by means of the preceding arrangement has its own independent supply of blood from the arterial system of the mother, the ovarian arterial trunk being a branch of the median dorsal aorta. Each egg and egg-sac is thus supplied with materials for its growth and maturation, the latter eventually becoming specialized into a contrivance by which the lives of the developing embryos are maintained while undergoing development in their respective follicles. The young or unripe eggs which are found together in the same ovary with the developing foetuses are, as stated above, enveloped in a cellular and fibrous stroma, which serves not only to strengthen the vessels, but also afterwards enters into the structure of the walls of the ovarian sacs or follicles externally, as these grow in size . . . [See fig. 4.]

The ova after developing a little way are each enclosed in a follicle or ovisac, *membrana granulosa* of Von Baer, or *membrana cellulosa* of Coste. As the egg develops there seems to be a space gradually formed about it in the same way as described by Wyman in *Anableps*. This space is filled with fluid, and in this liquid, which increases in quantity somewhat as development proceeds, the embryo Cyprinodont is constantly bathed. [See fig. 5.]

There is no trace whatever in the egg follicles of Gambusia of an independent egg membrane, such as is present in the ovary of all known forms of osseous fishes which spawn directly into the water . . . [See fig. 5.]

Kuntz⁵ (p. 184), in his study of the reproductive organs of *Gambusia*, claims, disagreeing with Ryder, that the ovary itself has an exterior investment which serves as an oviduct at its

posterior end connecting thus with the urinogenital sinus. Our observations indicate that there is an exterior investment of the ovary (fig. 6) and that there is also a functioning 'oviduct' (fig. 7) which conveys the embryos to the sinus when they have burst forth from the follicles. A better term than oviduct would be genital duct, since this organ conveys only larval *Gambusia* after they leave the follicular tissue.

The females chosen for examination in this study were of all sizes and were in all conditions of gravidity, carrying ova and embryos of all degrees of development. Each egg and embryo was examined under the microscope to eliminate any possible error as to the classification of the contents of the ovary as ova or early embryos. It is not difficult in this species to distinguish between a mature ovum and an egg fertilized within a few hours, especially after the fish has been preserved in spirits. The embryo appears, then, if in a blastodisc or blastoderm stage, respectively, as a white cap or a white streak of tissue. Not only are the embryos readily distinguishable by general appearance, but also, ordinarily, by their relative sizes. There is always a batch of minute ova embedded in the follicular tissue (fig. 4), but these are so small and opaque that they could never be mistaken for ova capable of fertilization. As the embryos of a large female, say, over 3.3 cm. in length, reach maturity and are liberated, there is a slight growth of the ova embedded in the follicular tissue; but in fishes of this and greater size, it is evident from our observations that these ova never attain full development, become mature, or are fertilized during the breeding season of that year.

There is, as has been said, especially in the large females, a considerable mass of embryos undergoing development, as Seale's⁸ (p. 181) data show. Fertilization, however, of all ova in females of appreciable size (3.3 cm. and larger), from our observations, occurs at approximately the same time. This conclusion is based on several facts.

In the first place, in females of appreciable size collected at any time from March 15th to October 1st, embryos are always found which are in very closely succeeding stages of development,

and never has there been found in the females examined a very or moderately advanced embryonic stage in the ovary simultaneously with a very early stage or with unfertilized but mature ova. Occasionally an abnormally developed embryo, embryos, or ova have been found. Abnormally developed ova and embryos have been observed in females examined in the proportions of one to twenty-six and one to seventy-five, respectively. The embryos were always malformed and the ova were always very few, the usual number being one or two. To cite typical cases: in a female measuring 3.85 cm. collected in September, 1917, one apparently abnormal ovum was found with thirty-three normal embryos; in a female 3.45 cm. in length taken in April, 1918, one abnormal embryo was found with eighty-two normal embryos, all in approximately the same stage of development. In seven of all females examined, ova measuring from 1 mm. to 1.4 mm. have been found with embryos in the same ovary. It is believed that this condition, being so different from that of the very large number of females examined, has resulted from an abnormal condition of the ovary or of the ova and does not signify a possible series of impregnations.

Again, if fertilization were not simultaneous for all the ova of a female's annual production, it would be expected that in some of the females examined during the year unfertilized but mature ova (a mature ovum measured 1.6 to 1.8 mm.) would be found at the same time as embryos. Such a finding would indicate the possible occurrence of waves of production of maturing ova and their subsequent impregnation and embryonic development, and would signify also that embryos in several well-separated stages of development would be found within the ovary of the same female. Such a condition, however, has never been found in the large number of females examined.

In fish examined which carried embryos, the ova which accompanied the pregnant condition were very minute, varying in diameter between the extremes 0.13 and 1 mm. (fig. 4), for the interval from March to February. These minute ova continue within these extreme measurements until about February 20th, when development begins, and in the largest fish, embryos in

early stages are found about March 15th. After fertilization the development of the embryos continues apparently simultaneously and in the same amount in each embryo until a rather late stage (fig. 8), from which point the final development is attained, in some earlier than in others. Those embryos which first obtain complete development are given birth and constitute the young of the first batch of the annual brood. The cause of the quickened development and of the resulting appearance of batches of the brood at intervals is problematical. This may, however, be due to the location of the embryos in the ovary. After the production of the first batch the embryos next to be liberated are given their final development and are liberated, and so on, until the entire annual brood is released.

Kuntz⁵ (p. 183), writing of the fecundity of *Gambusia* collected at Beaufort, North Carolina, states that—

In the same ovary may be found ova in various stages of development ranging from almost microscopic dimensions to a diameter of 1.8 millimeters attained at maturity. A considerable number of ova reach maturity at the same time. These being fertilized give rise to a brood of young. After the birth of this brood, another lot of ova reach maturity, and, being fertilized, give rise to a second brood. Thus, perhaps, all the ova required to produce the several broods which are born during a spring and summer may be present in the ovary at the beginning of the season.

The statement that mature ova are developed in waves of production and that liberation of a batch of young is followed by the fertilization of a batch of ova just matured, which will subsequently be the next batch of young liberated, does not find corroboration in our observations. However, we agree that all the ova which constitute the annual brood are in the ovary at the beginning of the season. Hildebrand⁴ (pp. 7 and 8), discussing the life-history of this minnow, points out that his experiments, carried on at Beaufort, showed that females, allowed to associate with males in the spring and then separated from them, continue to produce young fish throughout the season, during which at least five broods are liberated. He points out also that females allowed to associate with males through the summer and separated from them late in the fall

and then carried through the winter, did not produce young the following spring, and that the ova of these females were not fertilized. He concludes that the causes of these phenomena were the facts that spermatozoa could be carried by the female throughout the breeding season, but could not survive in the female during the winter period. His inference was based on an analogy between a description of the reproductive organs of certain other viviparous fishes of the same family, Poeciliidae, and his knowledge of those of *Gambusia*. More precisely, in the two viviparous forms which he mentions as related to *Gambusia* he says, quoting from Phillipi⁶—

Within the folds of the lining of the oviduct the sperms were found in great numbers, even after the birth of the young; . . . it is probable that the sperms are retained there throughout the breeding season and that the eggs are fertilized as soon as they are sufficiently matured.

Whether this supposed storage of spermatozoa occurs or not, the simultaneous fertilization of all the ova which will represent the year's subsequent production of young precludes the necessity for it. If there is storage, it would appear to be only another example of the excessive overproduction of the male sexual element and of the unnecessary frequency of copulation to insure maximum fertilization and survival of the species.

With further reference to the simultaneous fertilization of all the ova that may represent the year's subsequent production of young, the embryo counts have been divided into groups of collections occurring during periods of two months' time. They have also been averaged for each pair of lengths indicated, measurements being from the tip of the head to the base of the caudal fin (table 1). The number of embryos averaged and tabulated bi-monthly appears as shown in table 1.

The curves in figure 1, supplemented by the above discussion, indicate that female *Gambusia*, approximately 3.3 cm. and more in length and possibly those of smaller size, have all their ova, which result later in the season in young fish, fertilized simultaneously. Taking a concrete example based on averages computed from counts of the embryos of a considerable number of

TABLE 1
Seasonal Gambusia embryo count, 1917, 1918, 1921

	LENGTH														
	1.7-1.8	1.9-2.0	2.1-2.2	2.3-2.4	2.5-2.6	2.7-2.8	2.9-3.0	3.1-3.2	3.3-3.4	3.5-3.6	3.7-3.8	3.9-4.0	4.1-4.2	4.3-4.4	4.5-4.6
April and { Fish May { Embryos				1 17		10 19.6	17 24.1	16 31.6	18 40.6	15 63.1	16 108.1	15 128.4	15 145.6	7 184.2	3 196.6
June and { Fish July { Embryos		5 8.6	17 13.8	24 17.6	27 24.7	20 26.1	12 33.9	7 40.4	4 40.2	8 60.3	3 93.6	3 93.6	5 91.2	2 101	
August and { Fish September { Embryos	1 9	9 9.1	9 13.5	21 15.4	10 20.5	11 26.8	4 23.2	6 41.6	4 40.2	8 40.5	4 49.5	4 49.5			
October to { Fish February { Embryos	1 0	6 0	11 0	19 0	20 0	9 0	7 0	4 0	5 0	2 0	2 0	2 0			

females, the tabulated size 3.7 to 3.8 cm. is chosen as representative. From this it will be noted that in April and May the average number of embryos found in sixteen pregnant females was 108.1; for June and July, 60.3; for August and September, 40.5, and for October, November, December, January, and February, 0; in fact, in these five months there were no embryos found in females of any size. This, being true of all the females of appreciable size and being supplemented by observations previously discussed, indicates that all fertilization of the eggs occurred early in the breeding season, and immediately after copulation, with no needed storage of spermatozoa.

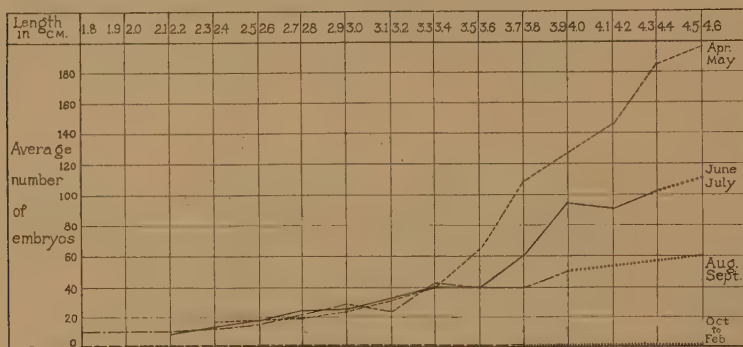


Fig. 1 Seasonal *Gambusia* embryo count. Estimated.

There is, therefore, in the case of *Gambusia* females of appreciable size a condition analogous to that found in many oviparous fishes, namely, that of having all the eggs capable of fertilization during the year fertilized simultaneously, and when these are laid or liberated no further production of young by the mother fish until the next year. This observation may or may not obtain for smaller females. Their very fast growth may possibly allow for two cycles of egg production within one year. It appears, then, from figure 1 that *Gambusia* females of appreciable size collected at Mound have but one annual cycle of egg production. This may hold true for smaller females, inasmuch as it has been noticed in this study that females of a size which would indicate that their birth occurred during the previous fall—that is, they

measured 2.4 cm. or less in length in March—neither carry embryos in March nor are their ova large enough to lead one to expect impregnation before at least six weeks. In no case were ova found in fishes of this size in March of greater diameter than 0.41 mm.

The relation of length of female to fecundity is best illustrated by the tabulated data for April and May (table 1). It will be noted that fecundity in this species increases markedly as the length of the female increases. From the average count of

TABLE 2
Gambusia frequency and varying influencing factors

	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
Gambusia frequency, 1919.....		13.7	9.9	12.1	22.5	39.2	52.2	122.9	69.6	38.5	20.0	23.1
Young frequency, 1919		0	0	4.0	5.0	10.2	10.2	20.6	15.8	2.6	1.3	3.2
Male frequency, 1919.		34.0	27.3	23.5	16.1	21.9	13.4	10.6	29.2	20.2	23.0	26.7
Average air temperature, 1915 to 1919, inclusive.....	46.4	52.1	59.5	63.9	71.0	78.4	80.3	79.8	73.4	65.7	55.4	48.5
Per cent of pregnant females 1917-1919..	0	0	35	?	40	58	73	81	69	0	0	0
Per cent of total liberation of young.....					31		62	100				

embryos based on April and May observations, the fecundity of females of 2.3 to 2.4 cm. length is doubled when the fish is about 3.2 cm. long; the fecundity is about quadrupled when the length 3.5 to 3.6 cm. is reached. With each 2 millimeters' growth thereafter the fecundity is greatly increased. The largest embryo count of this study was 226 for a female 4.3 cm. in length in May. The smallest female observed to be carrying embryos was 1.7 cm. in length and was carrying nine embryos in August.

From March through August there is a regularly increasing percentage of females that are carrying embryos. It appears that the proportionate number of females and males is such that

there is, at the beginning of the breeding season, a number of females that have not been impregnated. As the season progresses, however, the percentage of impregnated females increases, one copulation being sufficient for fertilization of all mature ova of that female. There is, then, a steady accumulation of pregnant fish during the spring and summer.

The percentage of unfertilized females in the spring and early summer is increased by those relatively few females born during the past late fall, which just reach sexual maturity and are

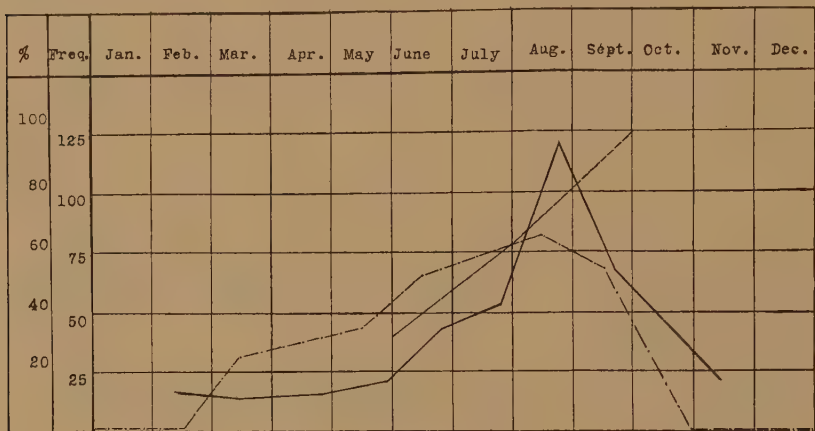


Fig. 2 Seasonal frequency in relation to fecundity in *Gambusia*. ———— *Gambusia* frequency; ----- Per cent of liberation of young; - · - · - Per cent of pregnant fish.

impregnated for the first time during the late spring. Figure 2 indicates the close relationship between the monthly percentage of pregnant fish and the changing '*Gambusia* frequency' curve of our recent paper¹ (p. 60). The highest frequency of *Gambusia* in our monthly observations at Mound occurred in late August. The highest percentage of pregnant females occurred at the same time. It appears, therefore, that the high frequency of *Gambusia* in midsummer results directly from the increasing percentage of pregnant females up to and including this time.

Realizing that the increased frequency of *Gambusia* at any time depends for the most part upon the prolificness of the larger females, especially since the production of young by the younger and smaller fish probably equals or merely balances the destruction of the species by natural causes, a correlation is noted between the bi-monthly record of liberated young (fig. 2), for a given size (the length 3.7 to 3.8 cm. being used as representative) and the monthly increase in *Gambusia* frequency. At the height of *Gambusia* frequency about 80 per cent of the year's offspring has been liberated.

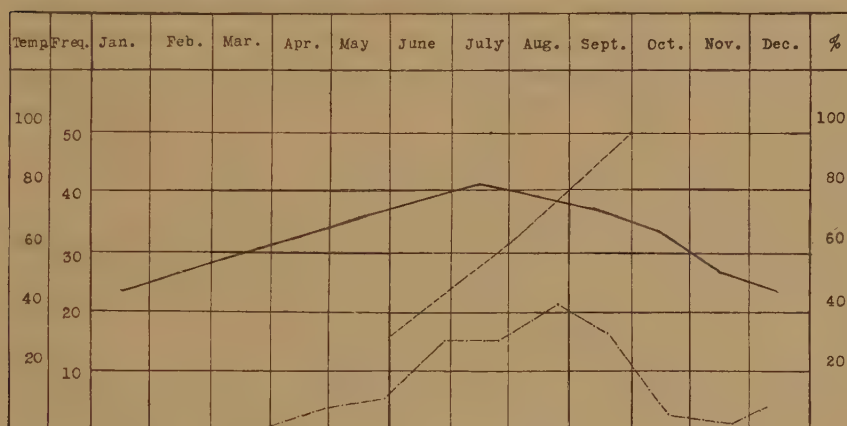


Fig. 3 Temperature in relation to fecundity in *Gambusia*. ——— Temperature of air; ----- Per cent of liberation of young; - - - - - Young frequency.

The seasonal liberation of young is dependent on the temperature of the water in which the fish live, other conditions being equal. The temperature record of table 2 is the monthly average mean temperature of the air as observed at Mound from 1915 to 1919, inclusive. There is no record available of water temperatures of all bodies of water concerned herewith. However, the close relationship of water temperature with that of the air for this vicinity is noted in a recent publication³ (p. 251, fig. 3). It appears that liberation of young is rapid through August, the last warm month of the summer, but that thereafter there is very little further liberation. In fact, as has been said, by the

1st of September at least 80 per cent of all births have been accomplished. Collections made after the 1st of October failed to reveal a single embryo. That there may be a few young born after October 1st is indicated, however, in the 'young frequency' of 1919 for the Mound vicinity¹ (p. 58, table 2).

After the last embryo has been liberated by the mother fish in the fall, even though the temperature is favorable for further development of eggs or for copulation and fertilization, development and impregnation of ova do not occur. Having collected young of the year as early as the middle of April, it is believed that the fertilization which initiated the development of these young must have occurred at least three or four weeks before this time. It is probable, in view of Seale's observations³ (p. 181) on the period of gestation, that fertilization among these fish occurred even much earlier than four weeks previous to the birth of the first young. The average mean temperature of the air at Mound for the month of March is 59.5°F., the same for October and November is, respectively, 65.7° and 55.4°F. It would appear probable that copulation and fertilization which may occur in March at the above-mentioned temperature could well continue at least until the 1st of November. The fact remains, however, that this does not occur. Metabolism of the fish cannot be markedly lowered by decreasing temperature or by lack of food before the 1st of November, since both of these factors remain favorable to this date. These facts suggest that temperature is of influence only in increasing the metabolism of the fish and is not significant in lengthening the breeding period. It appears, therefore, that egg production in *Gambusia* is a cyclical phenomenon, the annual quota of young being a certain number governed by the size and metabolic potentialities of the female for that season. The period from the first birth in April to the last in September or October is virtually a protracted period of gestation and parturition. The time intervening between the last liberation of one year and the fertilization of the next spring appears, then, whether temperature and feeding conditions are favorable or unfavorable for the maturing of eggs or the fertilization thereof, as a period of rest and recuperation for another

year's long period of pregnancy. This is readily suggested, also, by observations on the condition of ovaries during the period from October to January. During these months no ovum larger than 0.41 mm. in diameter was found in a total of sixty-three individuals examined. The modal size of these ova was 0.2 mm., and in each fish examined the ovary was very elongate and much reduced in size (fig. 9).

Referring again to the seasonal birth of offspring in this species (fig. 3), a positive correlation is noted between it and the 'young' of the previously mentioned paper on changing frequency in this species¹ (p. 67). 'Young' production, as observed at Mound, increased from late March to August, at which time the figures representing percentage of young liberated are at their highest. As might be expected, the percentage of pregnant fish is greatest in August.

SUMMARY

1. In addition to a tissue investing the ovary of *Gambusia affinis*, there is also a particular organ, the genital duct, which conveys the larval *Gambusia* from the ovary to the external sinus.

2. Evidence is cited indicating that the ova of all females of appreciably large size—3.3 cm. and more in length—in the vicinity of Mound have all their ova which appear later the same year as liberated young fertilized simultaneously.

3. Increasing size of females and coincident increasing fecundity of *Gambusia affinis* as observed at Mound, Louisiana, have been tabulated and discussed.

4. The relation of temperature of the water to the liberation of young and fecundity of *Gambusia* at Mound is indicated. It appears that 80 per cent of the annual production of young occurs before any considerable decline in the temperature of the water is noted.

5. Egg production in *Gambusia* is a cyclical phenomenon, the annual quota of young being a certain number governed by the size and metabolic potentialities of the female for that season, and not alone by temperature.

6. The seasonal rate of liberation of young *Gambusia* at Mound is positively correlated with seasonal abundance and with 'young frequency' of the species.

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PLATES

Photomicrographs (figs. 4, 5, 6, 7, 8, and 9) were taken by Mr. J. B. Southall, of the U. S. Biological Station, Fairport, Iowa.

PLATE 1

EXPLANATION OF FIGURES

4 Dorsoventral sagittal section of ovary containing developing embryos. Fish collected July 3, 1916; size, 3.5 cm. to base of caudal fin. Section shows developing embryos resting on yolks which float in follicular sacs. Near center of cut there appears a number of immature ova embedded in the follicular tissue. Stain, eosin. $\times 12$. Thickness, $50\ \mu$.

5 Dorsoventral median sagittal section of urinogenital system of a female *Gambusia* (same as no. 4), showing urinary canal, genital duct, and mouth of intestine. Lower embryo clearly shown floating in the follicular sac. Embryonic tail has been cross-sectioned. Stain, eosin. $\times 12$. Thickness, $50\ \mu$.

6 Dorsoventral section near median line (same as nos. 4 and 5), showing investment of the ovary and a broadened portion of the genital duct. Stain, eosin. $\times 7$. Thickness, $50\ \mu$.

7 Longitudinal section of non-gravid female collected March 10, 1921; size, 3.5 cm. to base of caudal fin. Section above the level of the colon showing folds of the genital duct, unfertilized ova embedded in the follicular tissue, and the urinary duct posterior to the genital duct. Stain, iron-alum haematoxylin. $\times 9$. Thickness, $50\ \mu$.

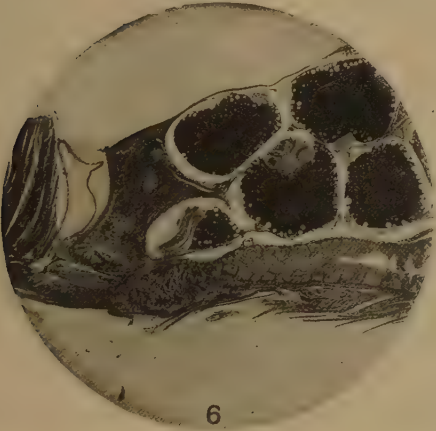
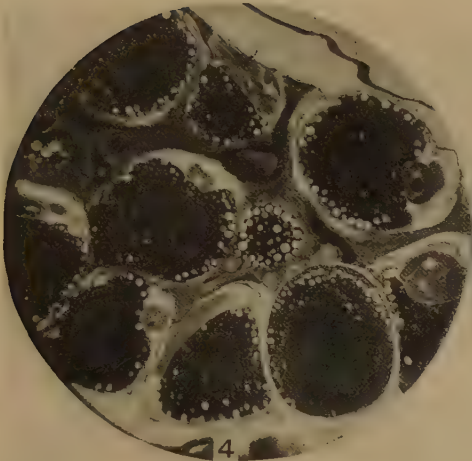
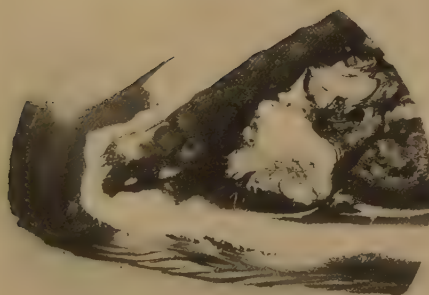


PLATE 2

EXPLANATION OF FIGURES

8 View of female, 2.75 cm. to base of caudal fin, collected September 13, 1918, with forty-one embryos dissected from the ovarian follicles. These embryos are all in approximately the same stage of development and represent probably the last two liberations of the annual brood. Approximately natural size.

9 Dorsoventral sagittal section of a female, 2.9 cm. to base of caudal fin, collected February 14, 1921, showing ovary in whose follicular tissue are contained numerous immature ova. Note the reduced size of the ovary and the very small ova. Stain, eosin. $\times 7$. Thickness, 50 μ .



Resumen por los autores, Carl Caskey Speidel y Roy M. Hoover

El método de la gelatina-glicerinada montada en un marco para la preparación y montaje de extensos cortes o de material patológico.

Mediante este método se pueden montar y conservar permanentemente extensas secciones de tamaño adecuado, tales como cortes transversales de un niño recién nacido, siendo de fácil manejo para demostraciones y estudio. Cada sección se coloca en una marco especial de madera, en el cual se ha fijado una placa de vidrio, que sirve de fondo del recipiente. El marco se llena con una mezcla de gelatina glicerinada y ácido fénico, que se vierte caliente, colocando el corte y cubriendo con una segunda placa de vidrio, que se fija por medio de listones de madera, completando de este modo el marco.

La gelatina se solidifica al enfriarse quedando incluida la sección en una masa de gelatina dura y transparente cubierta por las dos placas de vidrio y limitada lateralmente por el marco de madera. Los bordes de este se sumerjen en parafina fundida para evitar el acceso de aire. El material patológico puede también prepararse de este modo, así como secciones de cuerpos humanos jóvenes o de otros vertebrados de tamaño conveniente. Las principales ventajas del método son la eliminación de líquidos conservadores y evitar la posible mutilación o desintegración del material al manejarle.

Translation by José F. Nonidez
Cornell Medical College, New York

THE GLYCERIN-GELATIN PICTURE-FRAME METHOD OF PREPARING AND MOUNTING GROSS SECTIONS OR PATHOLOGICAL MATERIAL

1 CARL CASKEY SPEIDEL AND ROY M. HOOVER

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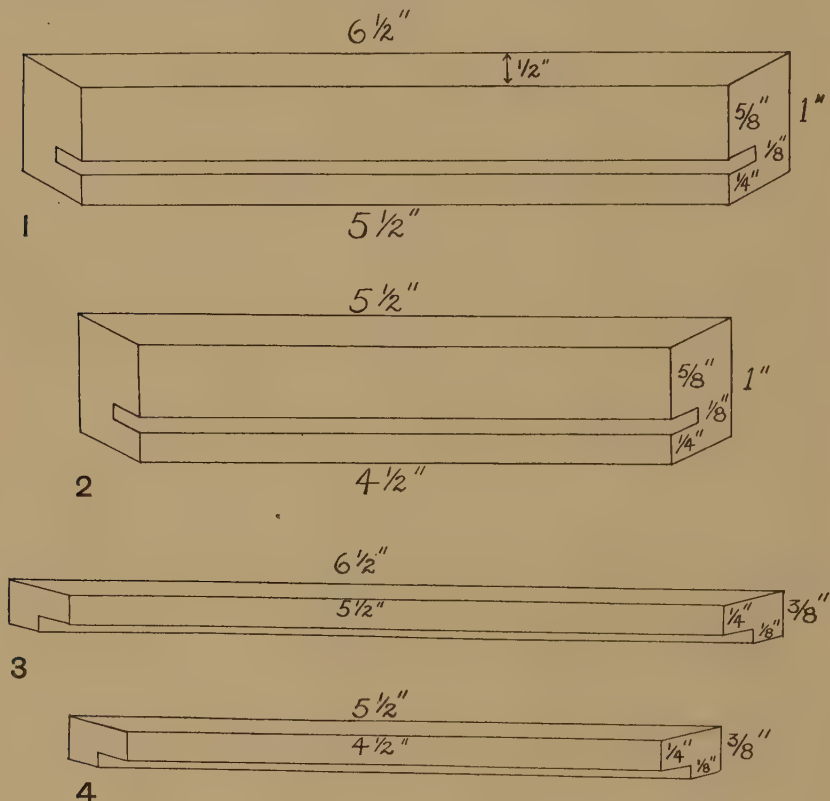
FOUR FIGURES AND ONE PLATE (FIGURES 5 AND 6)

Cross sections of the human body, such as are generally used for study in a course in topographic anatomy, do not last very long in good condition. Handling by the students inevitably results in the disarrangement and mutilation of the structures of the sections. The following method was used in an attempt to prepare sections in such a way that they could be kept permanently in first-class condition, but still be available at all times for study.

For this purpose an infant (which had died at birth) was used. Warm carmine-gelatin solution was injected into the blood-vessels by way of the femoral artery. This injection mass congealed as the body was cooled. The body was then placed in a jar of full-strength formalin after first injecting some of the formalin into the abdominal, cranial, and thoracic cavities to insure penetration. After a couple of days the body was sufficiently hard for sectioning. Sections were made by placing the body in a miter-box and cutting with a heavy, long, sharp knife. The cross-sections were made about $\frac{1}{2}$ inch in thickness.

The sections were now to be mounted in a warm glycerin-gelatin solution which would harden when cooled. For permanent preservation the mount must be made air-tight, in order to prevent drying of the gelatin. Special frames were made for this purpose. Two long strips of pine wood were obtained. Strip A was 1 inch wide, $\frac{1}{2}$ inch thick, and with a groove $\frac{1}{8}$ inch wide and $\frac{1}{4}$ inch deep, placed as in the diagrams (figs. 1 and 2).

Four pieces with mitered ends were cut from this strip; two were $6\frac{1}{2}$ inches in length (fig. 1) and two were $5\frac{1}{2}$ inches in length (fig. 2). These were nailed together to form a rectangular frame with a glass plate fitted into the groove, the whole resembling somewhat an ordinary picture frame.



Figures 1 to 4

In like manner four pieces of corresponding lengths were cut from strip B, which was $\frac{3}{8}$ inch wide, $\frac{1}{2}$ inch thick, and with a groove $\frac{1}{8}$ inch wide and $\frac{1}{4}$ inch deep, placed as in the diagrams (figs. 3 and 4). Together with another glass plate they formed the rest of the frame and were to be added after the cross-section and the glycerin-gelatin solution had been introduced.

Glycerin-gelatin solution was made up according to the following formula:

Water.....	42 cc.
Gelatin.....	6 grams
Glycerin.....	50 cc.
Carbolic-acid crystals.....	2 grams

The gelatin was soaked in water for one-half hour, then dissolved with gentle heat. Five cc. of white of egg were added and the mixture heated (the egg albumen gradually precipitating, carried down all fine particles of dust, etc., so that the gelatin was left perfectly clear). The gelatin was then filtered through moist cotton and the glycerin and carbolic acid added. The mixture was then warmed for fifteen minutes and stirred until homogeneous. (The gelatin should not be heated above 75°C., or it may not harden again at ordinary temperatures.)

A layer of this glycerin-gelatin solution was poured upon the first glass plate, then a section which had been previously dipped in the solution was laid upon it, then more of the glycerin-gelatin added until the frame was completely filled. The second glass plate was next fitted over the frame, all air being carefully excluded, and the four pieces from strip B nailed in their proper positions over the plate, thus completing the frame. The glycerin-gelatin mass hardened as the section was cooled. In this manner the section was completely embedded in hard clear gelatin mass, with a glass plate above and below and a wooden frame around the edges. It remained only to dip the edges of the frame in melted paraffin to make the whole mount absolutely air-tight and consequently permanent.

The photographs of plate 1 (figs. 5 and 6) show two views of a finished mount. Figure 5 represents an oblique side view. Figure 6 is a front view and shows a cross-section of an infant at about the level of the third lumbar vertebra. The photograph, however, gives little idea of the color contrasts that are displayed by the actual section.

This method could not be so easily applied to cross-sections of adult bodies, because of the size of the sections. The larger the glass plates are, the more easily they may be cracked or broken.

We believe, however, that for the smaller bodies of half-grown persons the method would be a very satisfactory one. Sections of adult human brains also make good material for mounting in this way. Good serial cross-sections of other vertebrate animals of suitable size (such as rabbits, cats, dogs, etc.), as well as of human foetuses, could also be obtained by this method. And finally, many gross pathological specimens could be mounted in this way in frames of suitable size and be conveniently available for purposes of demonstration or study.

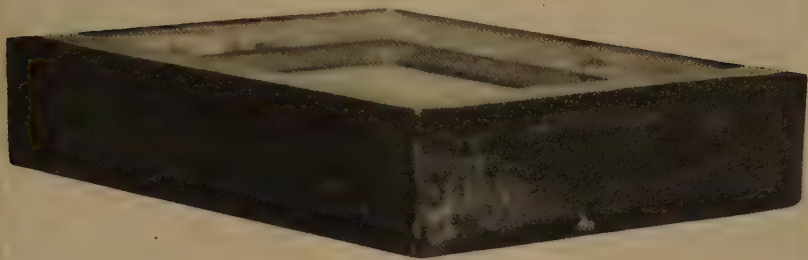
The elimination of preserving fluid and the elimination of the possibility of mutilation or distortion of the sections or specimens by handling constitute the chief advantages of this glycerin-gelatin picture-frame method.

PLATE 1

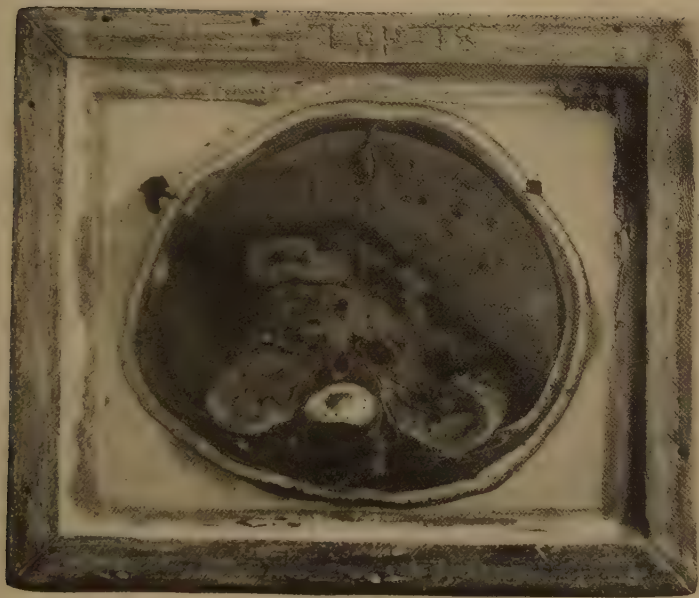
EXPLANATION OF FIGURES

5 An oblique side view of a finished mount, showing the structure of the frame. Photograph.

6 A front view of a finished mount, showing a cross-section through an infant at about the level of the third lumbar vertebra. Photograph.



5



6

Resumen por el autor, Ludwig A. Emge

Notas sobre el estudio de las mitocondrias en el amnios humano.

Los gránulos mitocondriales aparecen en las células del amnios humano en todos los estados de la gestación. Bajo el punto de vista morfológico son semejantes a las mitocondrias que se encuentran en otros órganos en todos sus rasgos, con excepción de su afinidad por las materias tintóreas, que es menor que la de las mitocondrias en otros tejidos. Su disposición está influida por la forma de la célula, pero es constante para grupos definidos de ciertos tipos de células. El periodo de gestación no influye sobre el tipo, forma, tamaño, disposición o número de las mitocondrias, con la excepción de los amnios muy jóvenes que el autor ha estudiado.

Translation by José F. Nonidez
Cornell Medical College, New York

NOTES ON THE STUDY OF MITOCHONDRIA IN THE HUMAN AMNION

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TWO FIGURES

The search for mitochondria in the human amnion was undertaken for purely anatomical reasons, in order to prove or disprove their presence and to establish such morphological variations which may facilitate future investigations. It is well known that certain types of these granules exist in other units of the gestation product. Van Cauwenberghe found mitochondria in the cells of the chorion and the placental villi during the first half of gestation. Later, Kervily demonstrated them also in the same structures during the second half of pregnancy, laying Van Cauwenberghe's failure to find them during the second half to insufficient chromization of the tissues studied. Kervily concluded that morphologically these granular structures were alike at the various periods of gestation. While studying the amnion I have had occasion to verify Kervily's findings, and can confirm his contention that mitochondria of quite similar appearance are present in the cells of the chorion and the placental villi at all stages of gestation. On the other hand, our own technical observations do not confirm Kervily's assumption that Van Cauwenberghe's failure was due to imperfect chromization, since we saw these granular structures equally often if chromization was materially shortened.

TECHNIQUE

The study of the material includes twenty human amnions fully mature and five younger amnions of $1\frac{1}{2}$, 4, 5, 7, and 8 months of age, respectively. Of the first group five were obtained

at caesarean sections before the membranes had ruptured. The location of the tissue removed from all membranes was carefully noted, and during the early part of the study various locations were studied in each amniotic sac. This latter procedure has proved to be of greatest importance, since there is a definite difference in the appearance of groups of cells within one amniotic sac. The greatest uniformity is present in the area covering approximately the inner two thirds of the placenta. For a comparative study of human amnions this is the only area which will give sufficiently uniform results to permit conclusions, and this rule for selecting tissues should be observed for all amnions more than five months old.

Since Kervily emphasized prolonged chromization of the tissues in order to arrive at the best results, small pieces of amnion obtained directly at birth were fixed in Regaud's neutral formaldehyde-bichromate mixture, which was changed daily for six days. The tissues were then chromicized in 3 per cent potassium bichromate for one week. (Osmic acid could not be obtained at the time of this study.) All tissues were collected in body-warm fluids and the containers kept in an ice-box for twenty-four hours. They were then transferred to a dark locker and kept at room temperature for the remainder of the period. The exclusion of light, and especially sunlight, is of importance in obtaining even penetrations with fixing agents containing potassium bichromate. I arrived at this conclusion by purely physicochemical reasoning. As a rule, such fixing fluids remain clear in the ice-box, and to some degree when light is excluded at room temperature, while, when they are exposed to bright light or sunlight or when they are kept in the incubator at 37°C., the fluids show a heavy precipitate. This means that certain rays and certain temperatures rapidly reduce the potassium bichromate. When this occurs, one finds a heavy water-insoluble layer around the tissue which prevents or materially retards the further process of fixing and mordanting. Since I have observed this occurrence, I have strictly adhered to fixing under the exclusion of direct light, and I am convinced that I have obtained tissues more uniformly penetrated by these fixing agents.

In the course of this study the period of fixing and chromicizing was gradually reduced to twenty-four and forty-eight hours, respectively, and it was found that this time is entirely sufficient to obtain satisfactory results. I, therefore, cannot join Kervily in his contention that Van Cauwenberghe's failure was due to an insufficient period of chromization. In this laboratory we practice secondary chromization as advocated by Bensley if we feel that poor staining results are due to imperfect mordanting. This method of chromicizing the mounted section for a few minutes before staining has proved very valuable in our hands. Nevertheless, staining difficulties will be encountered continually when it comes to staining the amniotic cell. This holds true for both vital and other stains. The only explanation that can be offered is that the plasma of this cell has a low chemical or physical affinity for the conventional fixing agents as well as for the mitochondrial stains. The prohibitively high price of other metals used in fixing fine cell structures has kept me from looking further into this question. The staining of the amniotic cell for mitochondria, either in the vital or fixed state, is a difficult problem indeed. I have not been able to discover the reason, but it remains a fact that this cell as well as all of its finer details have a poor affinity for dyes.

After trying out various methods, it was found that modified acid fuchsin mixtures, such as described by Altmann and by Bensley, would give the best results to demonstrate mitochondria, although even these 'best results' were not very satisfactory. It is of no use to describe the modifications, as they vary with each individual piece of tissue and depend upon the ingenuity of the morphologist in knowing how to adapt staining methods.

Vital dyeing was found to be unusually difficult. The vital dyes diffuse the cell plasma so quickly that it is often impossible to make out individual structures. To obtain a clear cell picture is a matter of luck in spite of the greatest care in not allowing the tissues to dry and in attempting to keep them at an even temperature. The saline solutions which were used as solvents for the dyes were varied in their composition, but this did not materially affect the end results. There was no better success when amni-

otic fluid was used as a solvent. After finding that all dyes related to neutral red and methylene blue were useless on account of their tendency to stain granules of various nature, Janus green B in 1:4000 Ringer's solution was used as a criterion. In tissues exposed to this dye, basal striations, apparently made up of mitochondria, could be seen occasionally in certain types of cells; in other types, masses of what seemed to be mitochondrial substance were observed. But the really finer types of granules could not be made out on account of the difficulty stated above. The only definite statement that one can make in regard to vitally stained amniotic tissue is that mitochondrial granules are present at all stages of gestation described here. A comparative study between fresh and fixed tissue beyond the question of existence of these granules in the amnion proved to be fruitless.

A STUDY OF THE FIXED MATERIAL

Among twenty-five amnions studied there were twenty mature membranes, five obtained at caesarean sections, before rupture, and one each from a pregnancy terminated therapeutically at 1½, 4, 5, 7, and 8 months. The outstanding factor is that there are several types of mitochondria in the cells of all these membranes, but that there is no essential difference in the morphological appearance of the individual types of granules in corresponding types of cells at the various periods quoted. A slight variation is found only on comparing the granules of the earliest to the fully mature membranes. While the relative number of mitochondria in the given cells of these membranes is apparently proportionally the same, one does notice a greater uniformity in the size and the shape of these granules as a whole in the earliest three amnions described here. The explanation for this may be the greater uniformity of amniotic cells as such at these periods. In the second half of gestation groups of cells within one amnion vary a good deal in appearance, which finding is most marked 'at term.' In the mature membrane there are found, side by side, groups of high and low columnar and cuboidal cells, each group varying somewhat in the detail architecture of its individual cells.

Nevertheless, after allowing for these slight variations, one can establish a mitochondrial criterion which is applicable to practically all groups of cell types in the individual membranes.

In the first half of pregnancy the globular and cocci-shaped mitochondrial granules are the most common forms present. In the second half rod-like and filamentous shapes become more conspicuous, and in the fully mature membrane masses suggesting mitochondrial substance are common. While the types of mitochondria are fairly uniform under similar conditions, the distribution of these granules varies within individual cells of the same type. Only in the earliest amnion studied a uniform distribution of the mitochondrial granules was found throughout the individual cell. In the cells of all other amnions the distribution depends definitely upon the shape of the cell and its location in regard to certain points of pressure.

I call attention to the fact that certain locations in the amnion do not represent the true general type of the amniotic cells and its internal architecture. For instance, each membrane has certain areas in which cell life is either interrupted or actually at its end, if one may judge from morphological appearances. The extreme of this trophic disturbance is best seen in an area varying from 8 to 10 or more cm. in diameter, which apparently represents the point of greatest amniotic pressure. Here the cell structures have lost most of their staining affinity. If one may use the expression, it is only the shell of the cell that accepts some dye. Usually the cells of this area are flattened out. Their nuclei are indistinct or necrotic. Mitochondrial structures are commonly absent or exceedingly scarce, and in the latter case will not hold the dye. My first impression was that this area was always located over the internal os of the cervix uteri, since it was found here in all of the unruptured membranes obtained at caesarean sections. That this assumption is not correct was demonstrated later by a comparative study, when it was found that this area may be situated at higher levels and that it corresponds to the site of the spontaneous rupture of the membrane. Usually there are several of these areas demonstrable which vary in degree of trophic changes. It is true that in multiparous

women such an area is commonly present over the internal os of the cervix, although this may not be the future point of rupture, and therefore at least two atrophic areas may be encountered in one membrane. In primiparous women the location of these areas may be found anywhere. The presence of these areas is constant in all membranes and suggests that the spontaneous rupture occurs at the point of greatest disintegration of cells. Since the outline of this area or areas is irregular, it was found advisable to secure tissues a good distance away from the point of rupture. The best and uniform tissues were obtained from that part of the amnion which covers the inner half of the placenta. I have dwelt so lengthily on this factor because if this rule is not observed future studies must end in conflicting observations.

The distribution of mitochondrial structures in the membranes of the second half of gestation is relatively uniform in corresponding types of cells, as pointed out above. Here one often notices a distinct bipolar distribution of mitochondria with a rather marked difference in the appearance of the granules at each pole. The largest amount of mitochondria is seen in the basal third of the cell regardless of what shape the cell may be. The closer the granular structures approach the actual base of the cell, the oftener they are seen to assume a formation of basal striation. This is most pronounced in the high columnar and piriform cells. This striation is made up of short parallel thin rods of equal length. The individual rods which bulge slightly at their midpoints rarely traverse more than one-sixth of the entire length of the cell and most commonly occupy about one-tenth of the long diameter of the cell. When the cell approaches the lower types of columnar shape, the striation begins to move away from the base. At the same time the staining intensity decreases and some of its definition is lost until, when the cell becomes very low, it is lost entirely. Mitochondrial structures are then replaced by a conglomerate mass that has all the staining characteristics, vital and fixed, of mitochondrial substance. Janus green B is taken up more readily by this substance than it is by defined mitochondrial structures. When exposed to any

of the mitochondrial solvents the mass disappears, which leads me to believe that it is either a massing of mitochondria prior to dissolution or that it is a direct and very early mitochondrial derivative. This mass also has a marked affinity for fat dyes

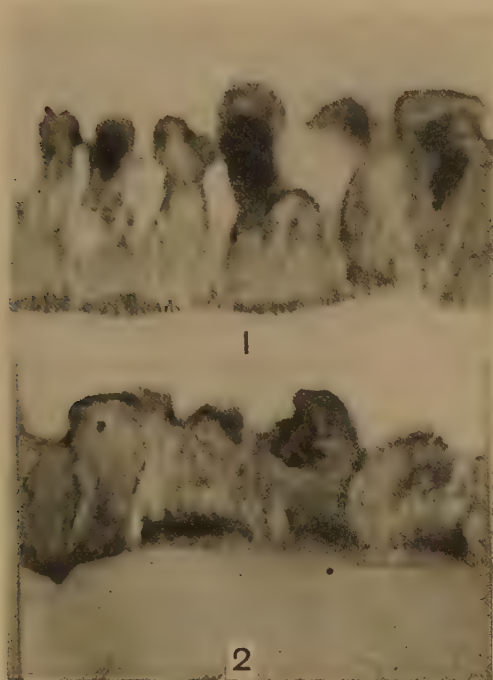


Fig. 1 Photomicrograph with oil-immersion lens. Represents the rod-shaped mitochondria of the columnar cell types. Note the regularity with which they are arranged in basal striation. The cupolas of the cells are connected by fine plasma bands. Few spherical mitochondria can be seen in several cell cupolas.

Fig. 2 Photomicrograph with oil-immersion lens. Represents the low columnar and cuboidal cell type in which the mitochondrial substance has lost its definition and forms a conglomerate mass away from the base of the cell.

about in the same proportion as mitochondria have when they undergo dissolution. Such masses are rarely seen in the high types of cells and are also uncommon in the very lowest types. Most commonly they are seen in the cuboidal and low columnar types.

Towards the mid level of the cell mitochondrial structures become very scarce. The only exception to this is an occasional very slender cell standing distinctly alone and supported only by very delicate plasma strands bridging to adjacent cells. In this type of cell long, slender filamentous mitochondria traverse the entire length of the cell. Individual filaments gracefully embrace the nucleus, leaving a very narrow clear zone in its direct vicinity, and enter the cell cupola to form a densely interlaced labyrinth. In all other cells there is, as a rule, a fairly wide nuclear zone which is free from mitochondria. This zone varies in size and position with that of the nucleus. It is widest if the long axis of the nucleus is parallel to that of the cell and narrowest if the two axes intersect at right angles. A fair appreciation of this can be gained only if the cell is seen in its true profile. Most often this zone occupies the upper end of the middle third of the cell, but it may actually move into the upper third and even enter the cell cupola.

While in the basal third of the cell rod-shape types of mitochondria are the outstanding structures with the globular and filamentous forms in the minority, the reverse is true of the upper third of the cell. In fact, here rods are so rare that they are negligible and filamentous forms occur only in the certain slender cells described. Most commonly, one finds the spherical, small, cocci-like types, but in contradistinction to mitochondria in the basal third these globular and cocci-like structures are ill-defined. They are few in number, vary considerably in size, usually are located in the joints of the cellular network, which renders them still more indistinct, and tends to form a very thin conglomerate layer directly under the membrane of the cupola. Occasionally, one sees strands of these conglomerates enter the intercellular bridges.

Aberrant forms of mitochondrial structures may be found in any type of cell, but their number appeared to be so small that it seemed to be insignificant to describe them. No attempt has been made at this time to make a very close comparison of the earlier amnions in regard to mitochondria, because the material is too scanty to obtain definite comparative pictures. The

description of these, therefore, has been confined to the most general statements made in the first part of the report.

CONCLUSIONS

In conclusion it is stated:

That mitochondrial granules occur in the cells of the human amnion at all stages of gestation;

That, with the exception of their staining affinity, which is less than in other tissues, they morphologically resemble in every respect mitochondria found elsewhere;

That their arrangement is influenced by the shape of the cell, but that this arrangement is quite constant for definite groups of certain types of cells;

That, with the exception of the very earliest amnion studied, the period of gestation has no influence on the type, shape, size, arrangement, or number of mitochondria seen.

I wish to express my appreciation to Miss Ella Wing, who looked after the cutting and mounting of the material studied.

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Resumen por los autores, J. A. Myers y Frank Myers

Estudios sobre la glándula mamaria.

VII. La distribución de la grasa subcutánea y su relación con las glándulas mamarias en vías de desarrollo del macho y la hembra de la rata albina desde el nacimiento hasta las diez semanas de edad.

El depósito de la grasa subcutánea es más marcado en la vecindad de los conductos laticíferos—formados y en vías de desarrollo. Desde el nacimiento hasta las diez semanas de edad, la grasa subcutánea se distribuye del mismo modo tanto en los machos como en las hembras. Los autores creen que el depósito sirve como protección contra el frío y como reserva alimenticia. La ausencia de una capa adiposa protectora distribuida uniformemente en la rata puede estar relacionada con el desarrollo precoz del pelo y la costumbre de los animales de vivir en espacios cerrados. En las ratas imperfectamente nutridas desde el nacimiento, el depósito de grasa subcutánea decrece y el desarrollo de los conductos laticíferos se retarda.

Translation by José F. Nonidez
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STUDIES ON THE MAMMARY GLAND

VII. THE DISTRIBUTION OF THE SUBCUTANEOUS FAT AND ITS RELATION TO THE DEVELOPING MAMMARY GLANDS IN MALE AND FEMALE ALBINO RATS FROM BIRTH TO TEN WEEKS OF AGE

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THREE FIGURES

While fixing the glands for fat stains in the study of witches' milk (Myers, '19), it was noticed that when the entire skin of an albino rat was removed, stretched out on cork, and placed in Flemming's fluid for a short time, the subcutaneous fat was beautifully differentiated from the surrounding tissues. There seemed to be a constant relation between the deposit of subcutaneous fat and the development of the mammary glands. Hence the present work was undertaken to determine this relation.

MATERIAL AND TECHNIQUE

Representative stages from birth to ten weeks were selected from apparently healthy albino rats of normal body weight. For each stage a male and a female from the same litter and of approximately the same body weight were studied and compared. The rats were killed by chloroform and an incision was made extending from the dorsal part of the root of the tail along the spines of the vertebrae and over the skull to the tip of the nose. The skin was then reflected, special precautions being used to remove all the subcutaneous fat with the skin. The skin was then stretched out on a piece of cork with the inner surface up, after which it was placed either in Flemming's fluid or 1 per cent osmic-acid solution. The preparations were left here until the subcutaneous fat had become dark brown. This usually required

thirty minutes to one hour. They were then washed in running water for an hour or so and carried through the various grades of alcohol. It was noticed that in skins left much longer than one hour the epidermis became somewhat darkened so that the subcutaneous fat was no longer so sharply outlined. After the material was hardened in the alcohol, drawings were made of the entire skin with the subcutaneous fat represented in black, the position of each of the twelve nipples being indicated on the drawing. These preparations did not show the ramifications of the milk ducts, hence it became necessary to remove the osmic-acid stain and make a study of the relation of the ducts to the fat. First, the connective-tissue layer of the skin was removed according to the method employed in an earlier work (Myers, '16). This thin layer, which contained the fat and milk ducts, was placed in a weak solution of hydrogen peroxide until the osmic-acid stain had entirely disappeared. It was then stained in very much diluted Delafield's hematoxylin and cleared according to the method described by Lane-Claypon and Starling ('06). These cleared preparations were then projected onto the original drawings in such a manner that the nipples coincided with the points indicating their position on the original drawing. This made it possible to outline the general distribution of each gland, thus showing the relation of the milk ducts to the subcutaneous fat.

Since osmic acid stains only the unsaturated neutral fat, triolein, the findings with osmic acid were verified by staining gross specimens with sudan III or scarlet red.

As soon as the skin was removed from the body of the animal, the entire body was placed in 1 per cent osmic acid to make sure that all subcutaneous fat had been removed. In most cases very little or no fat was left on the body wall of the animal.

In another series of animals the subcutaneous tissue was left on the body wall when the skin was removed. These bodies were then placed in osmic acid, scarlet red or sudan III, and the fat studied in its normal position. The results were similar to those with the other methods.

Microscopic sections were made through certain regions in order to verify the observations made upon the preparations stained in toto. Some of these sections were made from tissues fixed in Flemming's fluid. Frozen sections made from each stage studied were stained in sudan III or scarlet red and counter-stained in hematoxylin.

OBSERVATIONS

The stains employed failed to reveal any subcutaneous fat in gross preparations from animals killed immediately after birth. In specimens taken *six to ten hours after birth*, however, a considerable amount of fat is seen deposited in the subcutaneous tissue. This fat is not uniformly distributed. In gross specimens this subcutaneous fat does not appear in solid masses at this stage, but appears somewhat granular. In a general way it may be said that the fat is deposited in the area supplied by the larger arteries and their branches. Therefore, one finds fat deposited more abundantly in the inguinal region around the inferior epigastric artery and its rami and in the thoracic and upper abdominal regions around the superior epigastric and mammary and intercostal arteries. In the region of the ventral midline, and for some distance to the side of this line, no fat appears. In the midabdominal region where the superior and inferior epigastric branches become very small, fat is not deposited at this stage. The relation of this early fat deposit to the blood-vessels is very constant. Along the larger branches the fat is quite plentiful, but it gradually disappears as one passes along the rami of these branches.

The nipple of the abdominal mammary gland lies medial to the fat being deposited in the inguinal and lower abdominal regions. Its primary duct, however, passes caudolaterally and divides into two or three branches which enter the region of the depositing fat. Here these branches subdivide, and all the terminal ramifications of the abdominal gland are found in the fat deposit. The first and second inguinal glands lie medial to the posterior continuation of the region in which fat is being deposited. The terminal branches of their ducts, however, ramify in this fat.

In the cervical region is an area in which fat is being deposited that extends somewhat cephalad and laterad from the root of the neck. There is some indication of small strands of fat connecting this area with the thoracic area which extends caudad very nearly to the costal margin and dorsad where it closely approaches the midline of the back. The nipple of the first thoracic gland lies medial to the axillary region, and fat is being deposited between this nipple and the axilla. The primary milk duct from this gland passes cephalad where it enters, and its rami terminate around the fat being deposited. The second and third thoracic nipples lie medial to the fat being laid down in the thoracic region. The branches of these glands also terminate around this fat.

Thirty hours after birth the subcutaneous fat is present in greater quantities and extends over larger areas than in the preceding stage (fig. 1). In the inguinal and lower abdominal region the deposit of fat is so sharply outlined that it may be designated as the inguinal fat pad. This pad of fat on each side extends from a position anterior to the posterior portion of the crest of the ilium toward the midventral line. Before reaching this line, however, it turns caudad, passes over the pubis, and extends nearly to the anal region. That pad of fat in the region of the iliac crest has become so thick, and yet is so unevenly distributed, that in gross stained preparations transmitted light penetrates only in certain areas. However, as one passes toward the pubic region and caudad to this, one observes the fat in smaller amounts so that it has somewhat of a granular appearance. Extending from the pubic portion of this fat pad may be seen thin narrow strands of fat extending cephalomedially to a point near the umbilicus. At this stage the inguinal fat pads of the opposite sides are not connected. The inguinal fat pad does not extend medially as far as the abdominal nipple. In case of the first and second inguinal nipples, however, this pad of fat has extended very nearly to their lateral margin.

Cephalad to the inguinal fat pad is a considerable area of subcutaneous tissue which in the previous stage contains no visible fat. However, at this stage one sees an occasional very

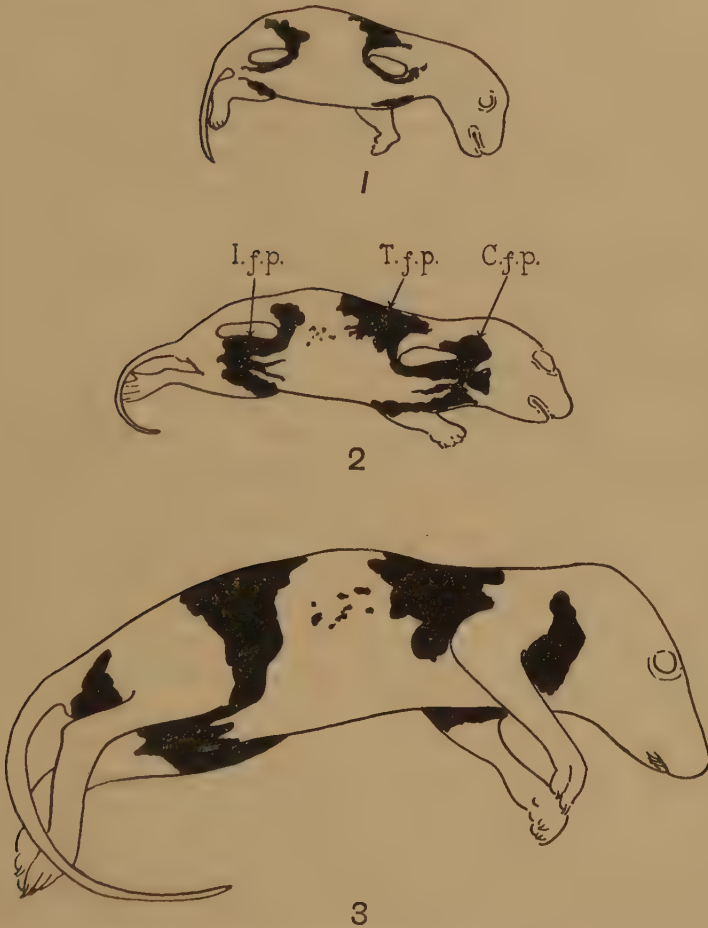


Fig. 1 shows the distribution of subcutaneous fat in an albino rat thirty hours after birth.

Fig. 2 shows the distribution of subcutaneous fat in an albino rat five days after birth. *C.f.p.*, cervical fat pad; *T.f.p.*, thoracic fat pad; *I.f.p.*, inguinal fat pad.

Fig. 3 shows the distribution of subcutaneous fat in an albino rat three weeks after birth.

small mass of fat in close relation to a blood vessel. As the thoracic region is approached, the subcutaneous fat is seen deposited in considerable amounts. In fact, one may now describe a thoracic fat pad. However, its boundaries are not so sharp as those of the inguinal fat pad. This is particularly true of the caudal boundary. The caudal boundary of the thoracic fat pad extends from a position very near the dorsal midline ventrally along and slightly below the costal margin. The medial boundary lies a few millimeters from the ventral midline, while the cephalic boundary passes just caudad to the axilla. This fat pad had increased in size so that its medial margin approaches very closely the lateral margin of the base of the nipples of the second and third thoracic glands.

The mass of fat being deposited in the cervical region in the previous stage has become quite definite in outline, and from this time will be designated the cervical fat pad. It extends from the anterior portion of the axilla into the root of the neck, where it turns laterodorsad and extends to the laterodorsal side of the neck. Caudally this pad of fat connects with the thoracic pad by means of narrow strands. The nipple of the first thoracic gland lies at the caudal end of the cervical fat pad, and its base is partly surrounded by this fat. The ducts of this gland ramify in the cervical fat pad.

Fifty-two hours after birth the fat pads are more distinct than in previous stages. They have not only thickened considerably, but extend over somewhat larger areas. The inguinal fat pads are slightly connected at their posterior ends. The cervical fat pads are also connected by small strands that extend across the midline. The inguinal fat pad has extended toward the midline until the nipples of the first and second inguinal glands are nearly surrounded by fat. The nipple of the abdominal gland still lies slightly medial to this pad. The strand of fat which in the previous stage extended from the pubic region to the region of the umbilicus has become more prominent. In the preceding stage attention was called to the fact that in the subcutaneous tissue lying between inguinal and thoracic fat pads an occasional small isolated mass of fat was observed. In the present stage such masses of fat are more numerous.

In the case of the nipples of the second and third thoracic glands somewhat isolated masses of fat may now be seen lying medial to their bases. The nipple of the first thoracic gland is so completely surrounded by fat that one is unable to see the position of the nipple in stained preparations. The cervical fat pads are slightly connected across the midline.

Five days after birth (fig. 2) the view of the bases of all the nipples except the abdominal, when examined from the inner surface of the skins, is completely obscured by the dense deposit of subcutaneous fat. In other words, the fat pads have extended toward the midline sufficiently to completely surround and cover up the bases of the nipples. In case of the nipple of the abdominal gland, the fat very closely approaches the lateral side of its base. The isolated masses of subcutaneous fat between the inguinal and thoracic pads have increased considerably in size. However, there is no direct fatty connection between these two pads at this stage. The cervical pads are now connected by good-sized masses of fat.

At the *end of the first week* the fat pads do not differ from the previous stage, except that they have become much thickened and slightly more extensive. The masses of fat between the thoracic and inguinal pads have increased in size so that in some specimens a few of them have fused to form a narrow connection between these two pads.

From the beginning of the *second week to the tenth week* (fig. 3) the subcutaneous fat pads increase in size with the general growth of the animal. They also vary somewhat in size depending upon the animal's health and food supply. During this period strands of fat may be observed extending across the midline connecting the cervical fat pads of opposite sides. Similar strands appear in the perineal region connecting the caudal portions of the right and left inguinal fat pads.

The milk ducts about the time of puberty undergo marked proliferation (Myers, '16) and it is interesting to note that their ramifications follow for the most part the same course of distribution as the subcutaneous fat followed at an earlier stage. Thus at this time milk ducts of the opposite sides cross the mid-

line in both the cervical and perineal regions and ramify in the fat already deposited in these regions. Other ducts send out branches which ramify in the processes of fat extending from the inguinal fat pads to the region of the umbilicus. Although strands of fat connect the thoracic and inguinal fat pads on each side, no ducts have been observed to ramify in these strands; so there is a definite interval existing between the ducts of the third thoracic gland and the abdominal gland on each side.

The ducts of the mammary glands of male rats after the fifth week do not grow as fast as those of females (Myers, '17), but no corresponding difference between the series was observed in the deposit of subcutaneous fat. The males at all ages show deposits similar to those of the females.

DISCUSSION

We believe neutral fat is deposited in the subcutaneous tissue for at least two purposes: first, as a protection against cold; second, as a reserve supply of food and energy. According to Berg ('11), much fat is deposited in the subcutaneous tissue of the new-born child. The question arises as to why so little or no subcutaneous fat is present in the rat at birth. In the first place, this animal is born in a very immature stage. Being born, however, without any special mechanism against cold, neutral fat begins to deposit beneath the skin very soon.

Again one might ask why this subcutaneous fat is not distributed more uniformly over the body. To be of much value as a protection against cold, one would expect to find a fairly uniform layer of subcutaneous fat as in some other animals. The answer to this may be found in the fact that the rat develops a fairly heavy coat of hair quite early in life; hence there is not much need for a further protective layer of fat. Moreover, the habitat of the animal may have some influence on the need of a protective layer of fat. For example, the rat usually inhabits fairly warm places, such as houses and cellars. It further protects itself by burrowing.

Attention has been called to the fact that the first subcutaneous fat is deposited in the neighborhood of the branches of the milk

ducts. Next, fat is laid down in greatest quantities in regions which will later be occupied by milk ducts. For example, the fat pads, at first small, become extended over a slightly larger area than the ducts will occupy during pregnancy and lactation. It was pointed out (Myers, '16) that the first cervical glands slightly before or at the age of puberty send branches across the midline so as to establish at least a gross continuity between the glandular tissue on one side with that of the opposite side. The same was true in case of the branches of the second inguinal glands. In both of these positions we see fat extending across the midline during the first week. In no other position at any time before puberty does fat extend across the midline.

Why should subcutaneous fat be deposited in definite pads in regions where the milk ducts later ramify? Is it because fat is a good medium for the ducts to proliferate in, or does it have some special rôle to play in the later functional stages of the milk ducts during pregnancy and lactation?

While studying the effects of inanition on the developing mammary glands (Myers, '19), it was observed that the subcutaneous fat that appears very early in the neighborhood of the milk ducts soon becomes greatly decreased after the amount of food is reduced to a minimum. In those animals that were underfed from birth very little subcutaneous fat was deposited. Along with this scanty deposit of subcutaneous fat occurs a partial failure of development on the part of the milk ducts.

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Resumen por el autor, John M. Tufts

Algunas observaciones sobre la estructura de las fibras de
Purkinje.

Al revisar la literatura sobre la estructura del sistema senoventricular, uno no puede menos de notar dos características: Primera, la escasez de la literatura, y segunda, las grandes diferencias de opinión con respecto do la estructura de dicho haz. Comenzando con la bien conocida descripción de las fibras por Purkinje en 1845 y continuando el exámen de la literatura hasta las últimas contribuciones de Van der Stricht y Todd (1920) puede notarse que no hay dos investigadores que hayan llegado exactamente a la misma conclusión relativa a la estructura del sistema. En la presente breve revisión de la literatura solamente se indican las principales diferencias notadas en las contribuciones.

Translation by José F. Nonidez
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SOME OBSERVATIONS UPON STRUCTURE OF THE PURKINJE FIBERS

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THREE FIGURES

In reviewing the literature upon the structure of the sino-ventricular system one is impressed by two characteristics: first, the paucity of the literature and, second, the wide differences of opinion regarding the structure of the bundle.

Beginning with the well-known description of the fibers by Purkinje in 1845 and continuing through the literature till the last contribution by Van der Stricht and Todd ('20), no two observers have reached exactly the same conclusion as to the structure of the system. In the present short review of the literature only the chief points of difference in the contributions will be noted.

At the end of his memoir, Purkinje described a network of gray, gelatinous fibers. These fibers were said to be applied to the internal wall of the heart and to pass through slits in the wall. Microscopically they were said to be composed of polygonal cells, shaped thus by reciprocal pressure. They were said to contain one or two nuclei. Between these cells a network of striated fibers not independent of the cells was said to exist. Speculating upon the physiology and histology of this apparatus, Purkinje finally concluded that it is muscular in nature.

Ranvier ('83) stated that the fibers of Purkinje are little translucent cords, anastomosing with each other and thus forming a reticulum, the meshes of which are very variable. In sheep, where there is no fat under the endocardium, the fibers are said to show less by their translucence, but appeared on the internal surface of the heart in light relief. On a slide they showed as

polygonal cells arranged in the form of a pavement epithelium. Following the branching of the bundle to the finest fibers, Ranvier found them to be composed of a single row of cells. The larger branches were composed of cells lying side by side and above each other. A transverse and longitudinal striation was found at the periphery of the cell and a granular mass of protoplasm which lay in the center had one or usually two nuclei.

The striation seemed to be continuous from cell to cell, but the latter could be separated by maceration with 40 per cent picric acid. The separate cells showed granular striations all around and pigment granulations in the protoplasmic center. The fibers were continuous with the cardiac musculature and the end fibers showed transition stages. Ranvier found them to have a connective-tissue sheath corresponding to a sarcolemma and regarded them as embryonic muscle fibers which had been arrested in development.

Schweigger-Seidel ('70), in his description of the heart as reported in translation in the Proceedings of the New Sydenham Society devoted to Stricker's Human and Comparative Histology, stated that

1. The transversely striated muscle of the endocardium occurs in two forms. Purkinje fibers or a wide-meshed network of muscular bundles, broader and shorter than the second group, or

2. Gray, gelatinous fibers described by Purkinje in 1845 as located under the endocardium. These Schweigger-Seidel regarded as a motor apparatus composed partly of an embryonic form of muscular tissue. According to him, these fibers unite to form meshworks and are composed of more or less prismatic segments 0.05 to 0.1 mm. in diameter with a cortical layer of transversely striated musculature and a hyaline axillary material containing one or two clear nuclei. Schweigger-Seidel stated that some observers regarded the transversely striated mass as an intermediate substance containing isolated cells, while others regard each unit as a muscle cell in which, as in a certain stage of development, only the peripheral layers have undergone conversion into contractile substance.

3. These fibers pass into ordinary muscle bands, and in some animals their place can be taken by muscle bundles.

4. A division of the stronger fibers does not occur, and they are surrounded by a connective-tissue sheath. When injected they form a wide-meshed network.

Lehnert ('68) expressed the view that the cells are masses of protoplasm contained within bundles of striations which are therefore *extra-* or *inter-* and not *intracellular*.

Retzer ('06-'08) stated that the Purkinje fibers differ markedly in various mammals. They are most like ordinary heart muscle in man and differ widely in animals such as the sheep. The structure of the bundle was also found to differ in various portions of the same heart.

The text-book descriptions of the bundle contribute little to the determination of its structure, being chiefly taken from the above and other descriptions. Poirier and Charpey ('12) followed Ranvier's views, but Testut ('11) regarded these fibers as cells of cardiac musculature arrested in development. Schäfer ('12) concurred in this, but in Piersol ('13) it merely is stated that "Their significance has only been recently recognized. They are now regarded as the terminal part of an elaborate system of special muscle fibers whose probable function is the coordinative connection of the auricular and ventricular musculature that otherwise are distinct and unconnected."

De Witt ('07) stated that the sinoventricular connecting system of the human heart is composed of the 'Knoten' and the main bundle which remains undivided for a distance of 11 mm. Division occurs at the point of union of the posterior and median flaps of the aortic valve and at the upper extremity of the ventricular muscular septum so that the right and left limbs are sub-endocardial. These limbs then branch and extend into various parts of the heart. Here they become Purkinje fibers, and as such are not composed of cells, but are syncytial in structure. De Witt's reason for arriving at this conclusion was that—

1. The fibrils and fibril bundles pass uninterruptedly through the fiber in different directions, forming a more complicated fibrillar network throughout the fiber than could be accounted

for by any other hypothesis than that the fiber is the unit of the system, and that—

2. White or elastic connective tissue was not found penetrating the fiber or dividing it except that a few peripheral strands seemed to do so, which appearances easily can be explained by the fact that the former follow the irregular contour of the fiber strand. Neither did blood vessels or nerves seem to penetrate the fiber bundles.

De Witt also saw the clefts mentioned by Tawara, but believed them to be due to shrinkage of sarcoplasm during fixation. The cell-like forms or sarcoplasmic territories were found to be very variable in form and size and were separated simply by strands of cross-striated fibrils.

Van der Stricht and Todd ('20) described the Purkinje fibers as containing three types of cells: 1) Polyhedral cells embryonic in type, with the long axis somewhat exceeding the rest; 2) cells of the adult type much longer than the foregoing, many of which are particularly bulky, and, 3) Purkinje cells transitional in type showing stages of transition between the Purkinje elements and myocardial cells.

They further stated that the Purkinje cells of adult type and those of transitional type are placed end to end to form Purkinje fibers, extending parallel to the axis of the atrioventricular bundle and being bound together by short oblique branches. The short cells of embryonic type were said to form a network, the fibers or trabeculae of which are much shorter and the meshes narrower. All these elements were said to be delimited laterally by the interstitial connective tissue which fills the spaces of the network. The ends of the cells were said to be separated by intercalated discs across which the myofibrils pass continuously from one cell to another. These transverse discs presented the same structure and the same significance as those of cardiac fibers, but were said not to traverse the entire thickness of the Purkinje cell. They were said to be formed by thick segments of myofibrils and by a special clear substance. Where these discs were seen from the surface it could be observed that the myofibrils were condensed into a system of thick bands which

anastomosed to form a network in the spaces of which a clear fluid existed. Van der Stricht and Todd could not say whether this liquid represents a part of the sarcoplasm or an intercellular substance, but they felt that the solution of this problem would permit us to decide upon the syncytial or cellular nature of heart muscle fibers.

Regarding the "Delimitation of Purkinje cells of adult type," Van der Stricht and Todd said: "We have already seen that cells of embryonic type are generally isolated laterally by an endomysial membrane and are placed end to end although separated from each other by an intercalated disc. This disc is always traversed by myofibrils continuous from one cell into the other. Precisely the same arrangement is found in the case of the elements of the adult type. . . . On account of this precise delimitation, the Purkinje fibers formed by the juxtaposition of cells resemble fibers of the myocardium in which, however, the vascular interstitial connective tissue is much more abundant."

Under the heading, "The sarcolemma of the Purkinje cells," they wrote: "We shall content ourselves with stating that the Purkinje cells possess no trace of a true cellular membrane formed by their cytoplasm but that they are circumscribed laterally by a membrane-like connective system or endomysium which often figures as a false festooned sarcolemma."

King ('15) thought that the system was composed of separate cells which could be isolated, and it was in order to follow up this idea that the following investigation was made. Professor Meyer suggested that if isolated cells could be teased out in their entirety as King held, and stained; thus revealing a complete and independent cellular structure, this would quite clearly demonstrate the cell as the true unit of structure of the system. In acting upon this suggestion, comparatively fresh tissue from hearts of bovines and sheep killed the same day or at most within the preceding twenty-four hours were used. If the material was over twenty-four hours old, difficulty was encountered in making preparations, as the separate cells could not be teased apart without destroying their integrity. The hearts were

opened after the method described by Knowler ('08) and the system well displayed. The Purkinje fibers are easily seen as described, especially on the papillary muscles and the false tendons.

Preparations were made also from human hearts. These resembled closely those made from the beef and sheep hearts, but since the material could not be obtained soon enough after death these preparations are not described, for they revealed nothing new.

The endocardium was raised with a pair of fine forceps and dissected from over a part of a Purkinje fiber. The latter was then dissected away from the cardiac musculature with a pair of fine needles and quickly removed to a watch-glass containing either glycerin or normal salt solution. In some cases tap-water was used.

The fiber was now teased out under a lens, mounted on a slide, and stained by drawing stain under the cover-glass. The stains giving the best results were dilute solutions of methylene blue, methyl violet and Delafield's haematoxylin and Van Gieson.

Maceration of the tissue in various reagents such as 0.5 to 2 per cent nitric acid, dilute sodium hydroxide and picric acid also was made use of. The nitric acid gave the best results. It made the teasing out of the separate cells easier and interfered the least with the structure and staining reactions of the cells. In using nitric acid, portions of the fiber were macerated for twenty-four hours, washed with distilled water, teased out, and stained as above.

Another method used was to stain portions of the fiber in Delafield's haematoxylin, pass them through graded alcohols up to 35 per cent strength and leave them in the latter for forty-eight hours. After this they were macerated in water for several hours and teased, giving good preparations. A series of over one hundred teased preparations of various parts of the bundle and its ramifications was made, and the following examples taken from my notes will serve to illustrate the results obtained.

Preparation I was taken from the left ventricle of a sheep's heart where a false tendon left the septal wall. Polygonal cells could be isolated by teasing in tap-water. Several single cells

were separated from the groups of cells detached by this treatment. On staining the preparation with dilute methylene blue what appeared to be cell membranes could be detected. Centrally located nuclei could now be seen in most cases and several double nuclei were observed. Nucleoli were present, being elongated in form.

Preparation II. Sheep's heart. A specimen was taken from the upper part of the right ventricle near the first branching of the bundle. It was teased out and stained with dilute methylene blue. Large polygonal cells were again observed in groups and as separate cells.

Preparation III. Sheep's heart. A specimen was taken from the anterior papillary network. Separate cells were demonstrable. Some of this tissue was macerated and treated with gentian violet. The cells stained very quickly and deeply.

Preparation IV. Beef heart. A specimen was taken from the anterior papillary muscle near the base. It was teased out in salt solution and stained with methylene blue. Separate cells were again demonstrable. These varied in size and shape from almost round to four- and five-sided cells. Two nuclei are present as in the fibers of the sheep heart. Large elongated nucleoli are present and stain darker than the nuclei. The nuclei take the stain more deeply than the granular protoplasm. They are centrally located in almost all of the cells. There appeared to be a definite cell membrane. When pressure was applied to the cover-glass the cells stuck together at first, but then separated, as if some intercellular cement were present. When separated the edges of the cells were smooth and showed no ragged outline or teeth suggesting the ends of torn fibrils as might at first thought seem likely.

Preparation V. Beef heart. A specimen was taken from the muscle of the left ventricle. The fresh teased preparation was stained in 2 per cent osmic acid. Separate cells were observed with distinct boundaries. The cytoplasm stained and was granular with a clear area at the periphery. No nuclei stained.

Preparation VI. Beef heart. A fresh teased preparation was made, stained in Delafield's haematoxylin, and left in 35 per

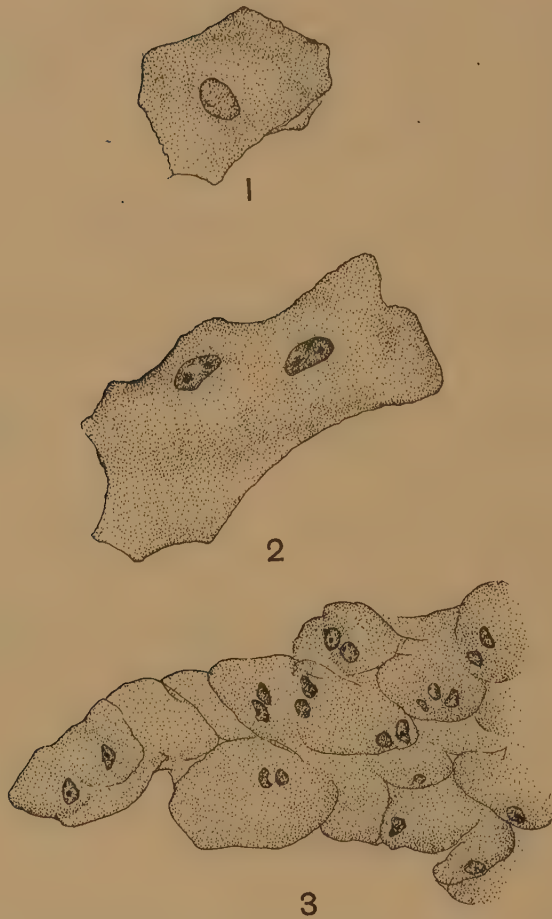


Fig. 1 Drawing of a single cell teased out from a section of Purkinje fiber taken from the papillary muscle in the left ventricle of a beef heart, stained with gentian violet. It shows the polygonal shape, granular cytoplasm with granulations more numerous at the periphery of the cell, nucleus and definite cell boundary.

Fig. 2 Drawing of a single cell from the same preparation as figure 1. Two nuclei are seen with nucleoli. The cell has a definite cell boundary.

Fig. 3 Drawing of a group of cells from a portion of a Purkinje fiber taken from the wall of the left ventricle of a beef heart. Partially teased out in tap-water and stained with gentian violet. It shows several cells with two nuclei and the granular cytoplasm especially marked at the periphery of the cells.

cent alcohol for forty-eight hours. It was then macerated in water and showed typical cells with what appeared to be a membrane and nuclei and nucleoli in each cell. Many cells possessed two nuclei and had a granular appearance.

In teasing out separate cells it was noted that the portion of the fibers used for teasing were in the form of circular rods composed of these cells. If a dissecting needle was rolled over this rod so as to break off a portion of the fiber, fibrils could be observed projecting from the torn ends of the rod. These fibrils showed cross striation upon staining with Van Gieson.

In an effort to ascertain the relationship of these fibrils to the cells several series of sections were made of pieces of the Purkinje fibers taken from the papillary muscle of beef heart. This material was fixed in Zenker's solution and imbedded in paraffin in the usual manner. Sections were cut both parallel to and at right angles to the long axis of the fiber from 2 to 4 μ in thickness and stained with Delafield's haematoxylin, counterstained with eosin or Van Gieson.

From these sections nothing definite as to the structure of the Purkinje fibers could be obtained other than what has been described by previous observers. Definite cells could not be made out in the sections. With a Van Gieson counterstain areas could be seen, with double nuclei containing an elongated nucleolus, in an area of clear cytoplasm and surrounded by an area containing striations seemingly continuous with those in the next cell. However, in the mounted sections no distinct division could be made out between the cells nor could the fibrils be demonstrated as being extracellular.

The preparations here described were duplicated many, many times and can easily be duplicated by anyone following the simple technique given. From the evidence so obtained it seems to me that the conclusion is justified that the cell is the unit of structure of the system. This cell, although large, varies greatly in size, however, in the same heart. The isolated cells appear to have a definite cell membrane surrounding them and vary in shape from almost spherical to rectangular or polygonal in form, suggesting the original statement of Purkinje that they are shaped

by reciprocal pressure. The cytoplasm is granular, the granules staining black with osmic acid. As observed by several investigators, many of the cells have two nuclei which are located centrally. These nuclei contain large, elongated nucleoli staining with ordinary nuclear stains, such as Delafield's haematoxylin.

From my observations on these cells in fresh teased preparations, I do not believe them to be embryonic muscle cells arrested in development.

In conclusion, the writer wishes to express his thanks to Doctor Meyer for suggesting the subject of this communication and for his assistance in carrying it to conclusion.

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Resumen por el autor, Wayne J. Atwell

La morfogénesis de la hipófisis en los anfibios caudados.

El autor ha estudiado la hipófisis en *Amblystoma*, *Spelerpes*, *Necturus* y *Amphiuma*. La hipófisis epitelial se desarrolla a expensas del ectodermo, diferenciándose en tres lóbulos: la parte anterior propia, la parte intermedia y la parte tuberosa. La parte anterior propia o lóbulo anterior propio, forma la mayor parte de la glándula y viene a rodear las superficies caudal y ventral del infundíbulo. La parte intermedia se desarrolla a expensas del extremo dorso-caudal del fundamento hipofisario primitivo. En su posición adulta está situada posteriormente al lóbulo neural, penetrando entre este último y el lóbulo anterior. La parte tuberosa procede de un par de procesos que crecen hacia delante desde el resto de la glándula. Estos procesos no se separan para formar placas epiteliales separadas como sucede los anuros, sino que conservan su relación con el lóbulo anterior durante la vida del individuo.

En *Necturus* y *Amphiuma* el lóbulo anterior está considerablemente dilatado. A juzgar por estos hechos solamente, estas dos formas son las primitivas en los anfibios caudados y están bastante íntimamente relacionadas con ciertos peces.

Translation by José F. Nonidez
Cornell Medical College, New York

THE MORPHOGENESIS OF THE HYPOPHYSIS IN THE TAILED AMPHIBIA

WAYNE J. ATWELL

Laboratories of Anatomy, Medical Department of the University of Buffalo

NINETEEN FIGURES

The pars tuberalis has been recognized as a division of the epithelial hypophysis quite distinct from either the anterior lobe proper or the pars intermedia only within the past few years. At present the developmental and adult relations of the pars tuberalis are well known for a number of the vertebrate classes, viz., mammals, birds, reptiles, and to a certain extent for the amphibia, due to the researches of Tilney, Bolk, Woerdeman, Baumgartner, Parker, the writer, and others. The question as to the presence of an homologous lobe in the hypophysis of the remaining vertebrates is one which must be settled before broad generalizations may be made with assurance. The homologies attempted by Woerdeman, for example, seemed premature, since he did not have at his command the developmental histories of the gland for the amphibia or the teleost fishes.

In a previous paper (Atwell, '18 a) the writer has described the development of the hypophysis in the anura. In these forms the pars tuberalis is developed as a pair of buds which grow nasalward and, at about the time of metamorphosis, become detached from the remainder of the epithelial portion to form two discrete epithelial plaques. A preliminary study of the development of the gland in the tailed amphibia showed certain differences to exist, one in particular being of sufficient interest to warrant a more detailed investigation.

PLAN AND METHODS

It has been the plan of the present study to follow the development of the hypophysis by close stages in one genus of the urodeles and then to compare with this certain larval and adult

stages from other of the tailed amphibia. For the former *Ambystoma* was chosen. Fertilized eggs of the species *A. punctatum* were collected and embryos and larvae taken and preserved at frequent intervals. Older specimens were of *A. punctatum*, *A. tigrinum*, and *A. jeffersonianum*. For comparison, larval and adult stages of *Necturus maculosus*, *Spelerpes bislineatus*, and *Amphiuma* means were studied.

The fixative most often employed was Bouin's fluid, but for certain of the younger and a few of the older stages formol-bichromate or corrosive sublimate was used. In the case of the *Amphiumae*, vascular injection was employed in fixation, preceded by physiological saline solutions to remove the blood corpuscles. Series of sections prepared and utilized for this study total twenty-eight series of *Ambystoma*, one of *Necturus*, three of *Spelerpes*, and two of *Amphiuma*. Free use was made of the Born wax-plate method of reconstruction to demonstrate the successive stages in the morphogenesis of the gland. Models were constructed at a magnification of 200 diameters for the larval stages and of 100 or, in one case, 50 diameters, for the adult stages. The drawings were made at the original magnification and have been reduced in most cases one-half off. The magnification given with the figures indicates their present actual size.

Acknowledgment is due to Miss Ida Sitler, formerly of Smith College, now of Hollin's College, Virginia, for the preparation of a number of the wax-plate reconstructions. I wish also to express my sincere thanks and appreciation to Mrs. H. H. Wilder, of Smith College, for the specimens of *Spelerpes bislineatus* which she kindly collected and fixed for me; likewise to Prof. Irving Hardesty for suggestions and aid in securing the specimens of *Amphiuma*.

OBSERVATIONS

a. Development of the hypophysis in Ambystoma

4 to 6.5-mm. embryos of *Ambystoma punctatum*. At the 4-mm. stage the hypophysis fundament is already well formed. In sagittal sections it is evident as a solid, wedge-shaped mass

of cells extending dorsalward from the inner layer of the ectoderm at the cranial end of the oral pit. It lies between the wall of the neural tube and the foregut.

By the time the embryo has attained a length of 5.5 mm. the hypophysis has become more elongated and is more tightly wedged between the brain wall and the foregut. Mitotic figures are present and the total number of cells composing the gland has increased considerably. Neither this stage nor the preceding gives satisfactory evidence to confirm a possible bilateral origin for the gland such as has been described by Kingsley and Thyng.

A 6.5-mm. embryo shows the gland united to the ectoderm by a stalwart connection. The oral plate is intact.

7.5-mm. embryo of A. punctatum. An approximately mid-sagittal section of the hypophysis region from a 7.5-mm. embryo is shown in figure 1. The epithelial stalk, by which the gland maintains its attachment to the ectoderm, has become a slender cord of cells. The dorsal end of the gland is enlarged and club-like. It presses tightly against the caudal termination of the diencephalic wall. The notochord, at this stage, does not extend so far cephalad as in a corresponding stage in the frog tadpole, and consequently its cephalic end is not in intimate relation with either the infundibulum or the epithelial hypophysis. The oral plate is intact.

9- and 10-mm. larvae of A. punctatum. Figure 2 A shows a wax-plate reconstruction of the hypophysis from a 9-mm. larva viewed from the ventral surface, with the caudal end below. The gland is just losing its connection with the ectoderm as its drawn-out cephalic end indicates. The detachment takes place close to the gland in such a manner that a considerable stalk is left attached to the epithelium. This is apparently retracted into the epithelium or else completely disappears. I have never noted in the Amphibia a separation of the stalk close to the ectoderm with the consequent formation of remnants, such as frequently are seen in mammals, and which may give rise to a 'pharyngeal hypophysis' or a 'parahypophysis.' There is a certain rearrangement, with more compact grouping, of the cells

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which are to form the pars intermedia. These are situated at the caudal free extremity of the gland.

12-mm. larva of *A. punctatum*. A wax-plate reconstruction from a larva of this stage is shown in figure 2 B. Comparison of A and B of figure 2 shows that after the detachment of the gland

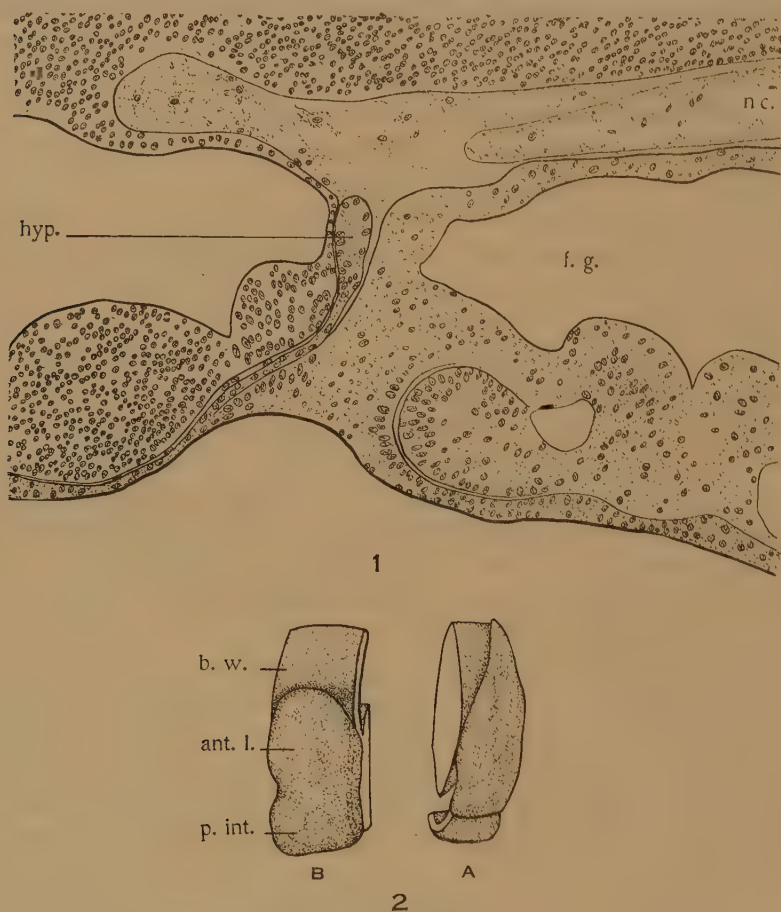


Fig. 1 Approximate midsagittal section of hypophysis region from 7.5-mm. larva of *Ambystoma*; nasal end at left. *hyp.*, epithelial hypophysis; *f. g.*, foregut; *nc.*, notochord. $\times 100$.

Fig. 2 Wax-plate reconstructions of the epithelial hypophysis and adjacent brain wall viewed from the ventral surface. *A*, from a 9-mm. larva of *Ambystoma*; *B*, from a 12-mm. larva. Caudal end below. *b. w.*, brain wall; *ant. l.*, anterior lobe; *p. int.*, pars intermedia. $\times 100$.

from the epithelium the hypophysis becomes both relatively and absolutely shorter. The cephalic end of the gland becomes blunt and round. It appears as though some traction which previously had kept the gland drawn out and elongated had been rather suddenly relieved. The pars intermedia is more distinctly differentiated and its separation from the anterior lobe proper is indicated by grooves at the sides.

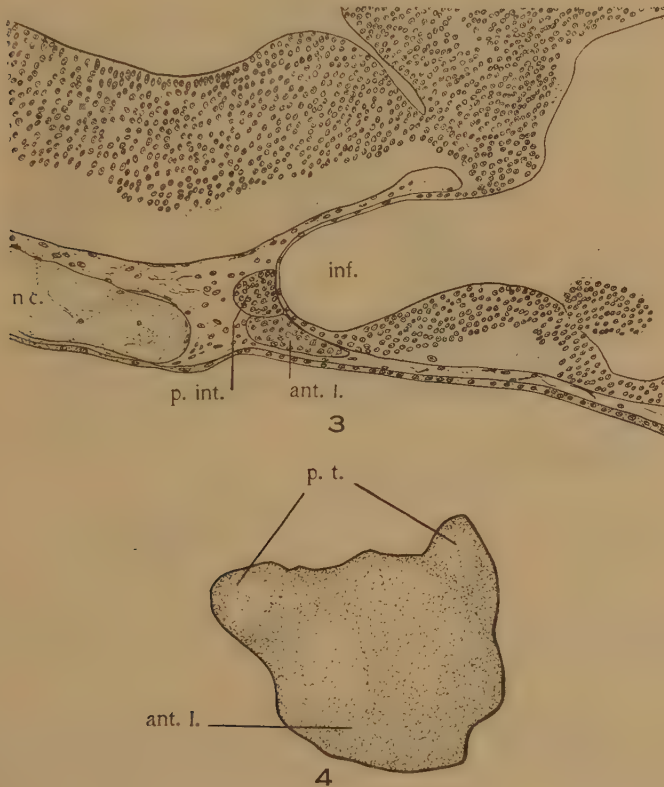


Fig. 3 Sagittal section of hypophysis region, 15-mm. Ambystoma larva. Nasal end at right. *nc.*, notochord; *inf.*, infundibulum; *ant.l.*, anterior lobe; *p.int.*, pars intermedia. $\times 100$.

Fig. 4 Ventral view of a wax-plate reconstruction of the epithelial hypophysis from a 38-mm. Ambystoma larva; caudal end below. *p.t.*, pars tuberalis; *ant.l.*, anterior lobe. $\times 100$.

14- and 15-mm. larvae of *A. punctatum*. Between the 12-mm. and 14-mm. stages the rupture of the oral plate occurs. Transverse sections of the hypophysis of 14-mm. larvae show a thin shelf-like lateral extension on each side of the anterior lobe proper. These are doubtless the lateral lobes which later form the pars tuberalis. A midsagittal section of the hypophysis region from a 15-mm. larva is given in figure 3.

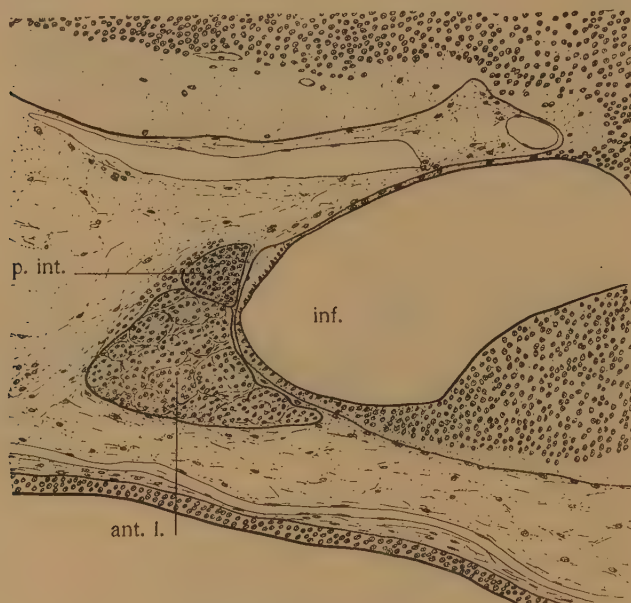


Fig. 5 Midsagittal section of the hypophysis region from a 40-mm. *Ambystoma* larva; nasal end at right. *inf.*, infundibulum; *p.int.*, pars intermedia; *ant.l.*, anterior lobe. $\times 100$.

38-, 40-, and 60-mm. larvae of *Ambystoma*. Figure 4 shows a ventral view of a wax-plate reconstruction of the epithelial hypophysis from a 38-mm. larva. The two buds which compose the pars tuberalis are well differentiated. The pars intermedia cannot be seen from this surface. The reason for this is well illustrated by figure 5, which gives a midsagittal section from a 40-mm. larva. The anterior lobe is molded around the caudal end of the infundibulum in such a manner that, with

the pars intermedia, a cup-shaped structure is formed. The pars intermedia is situated dorsal to the anterior lobe and at some distance forward from its caudal extremity. The neural lobe is apparent as a thickening of the part of the infundibular wall adjacent to the pars intermedia. The two bud-like portions of the pars tuberalis grow forward, becoming more elongated (fig. 6). The 60-mm. larva shows a considerable increase

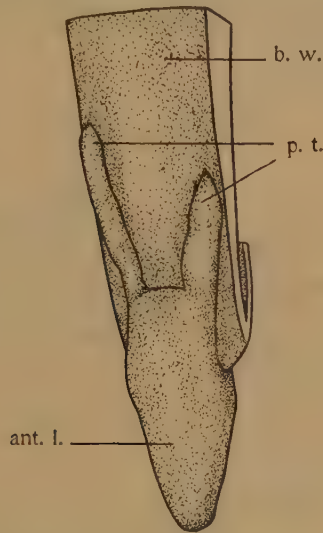


Fig. 6 Ventral view of a wax-plate reconstruction of the hypophysis from a 40-mm. larva of *Ambystoma*. Caudal end is below. *b.w.*, brain wall; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe. $\times 100$.

in the size of all parts of the gland, but the enlargement of the anterior lobe proper is especially apparent.

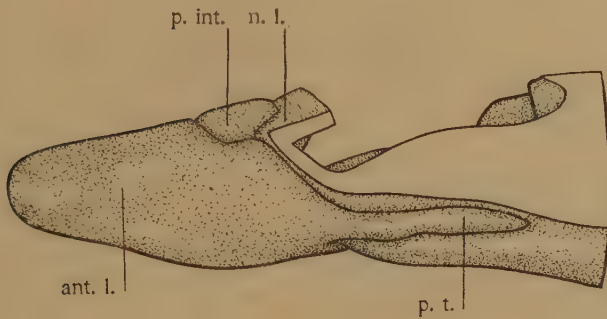
Adult Ambystoma jeffersonianum. The hypophysis of the adult ambystoma is shown in sagittal section in figure 7. Three views of the wax-plate reconstruction are given in figures 8, 9, and 10. The anterior lobe proper is seen to be the most caudal portion of the gland. The name 'anterior lobe,' therefore, has not been applied because of the relative position of the part in the adult amphibian, but because of its homology to the corresponding lobe in the higher vertebrates. Both sections and

reconstructions show that the pars intermedia is small. Its greatest dimension, which is from side to side, is less than the width of the anterior lobe proper. It is located dorsally and lies wedged in between the neural lobe and the anterior lobe (figs. 7, 8, and 9).

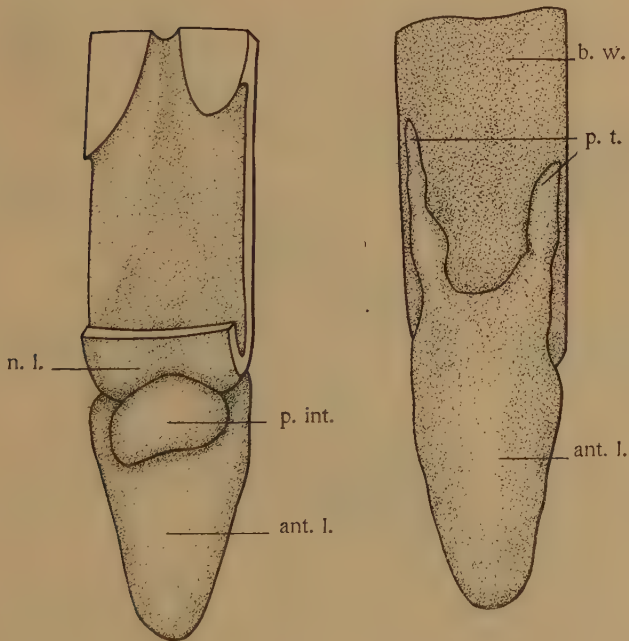


Fig. 7 Midsagittal section of the hypophysis region of an adult *Ambystoma*. The nasal end is at the left. *inf.*, infundibulum; *p. int.*, pars intermedia; *ant. l.*, anterior lobe. $\times 40$.

The adult condition of the pars tuberalis resembles very closely the larval. As shown by figures 8 and 10, the two processes of the pars tuberalis maintain their connection with the main body of the gland. They are elongated and lie embedded in the pia mater close to the floor of the brain. These processes do not become detached, as in the frog and the toad, to form separate epithelial discs, or plaques, but remain throughout adult life as two tongue-like processes extending nasalward from the anterior lobe proper.



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Figs. 8, 9, and 10 Wax-plate reconstruction of hypophysis and adjacent brain wall of an adult *Ambystoma*. Figure 8 views it from the right side; figure 9 is a dorsal view; figure 10 is a ventral view, caudal end below. *n.l.*, neural lobe; *p.int.*, pars intermedia; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe proper. $\times 50$.

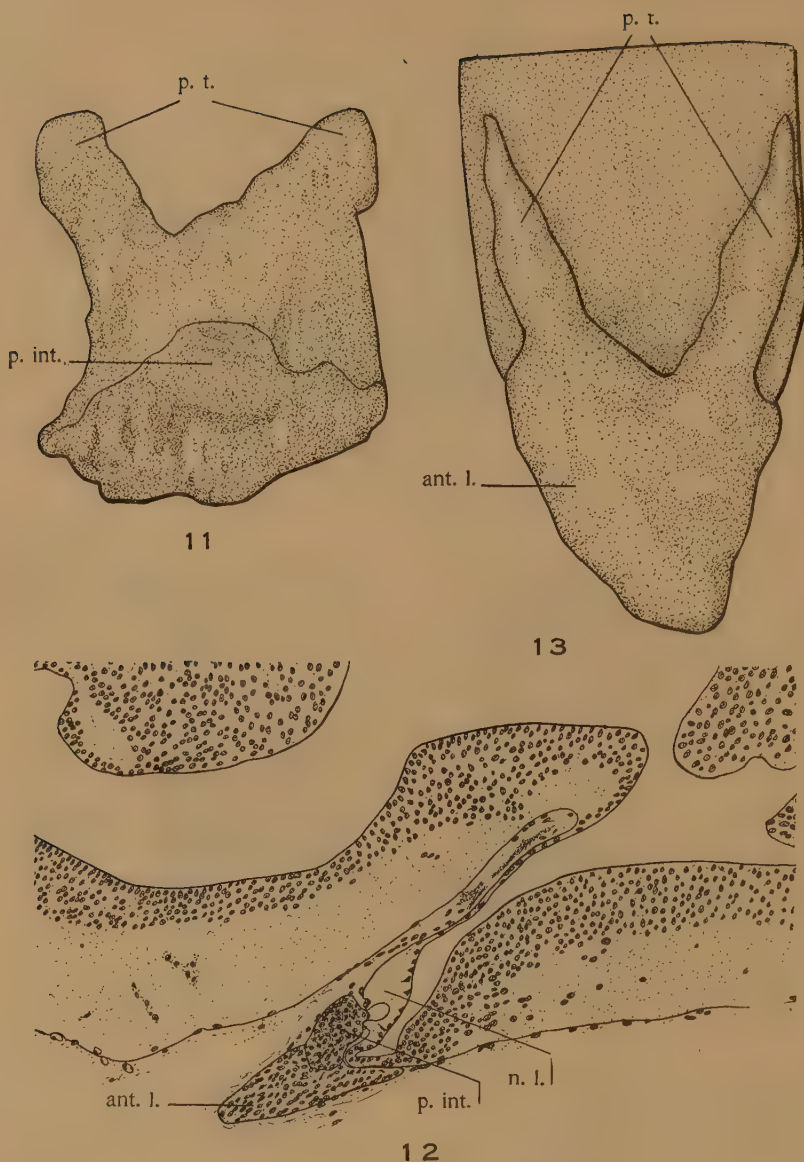


Fig. 11 Dorsal view of a wax-plate reconstruction of the epithelial hypophysis from a 19.5-mm. *Spelerpes* larva, caudal end below. *p. t.*, pars tuberalis; *p. int.*, pars intermedia. $\times 100$.

Fig. 12 Midsagittal section of the hypophysis region from an adult of *Spelerpes bislineatus*, nasal end at right. *ant. l.*, anterior lobe proper; *p. int.*, pars intermedia; *n. l.*, neural lobe. $\times 100$.

Fig. 13 Wax-plate reconstruction of the hypophysis of an adult *Spelerpes bislineatus*; caudal end below. *p. t.*, pars tuberalis; *ant. l.*, anterior lobe proper. $\times 100$.

b. The hypophysis of Spelerpes

For comparison the hypophysis was studied in three other tailed amphibia, with special attention given to the adult morphology of the gland. The forms chosen were *Spelerpes bislineatus*, *Necturus maculosus*, and *Amphiuma means*.

The specimens of *Spelerpes* include a 19.5-mm. larva, a larva of the 'pre-metamorphic stage' (Wilder), and an adult. A dorsal view of a wax-plate reconstruction of the epithelial hypophysis from the younger larva is shown in figure 11 and from

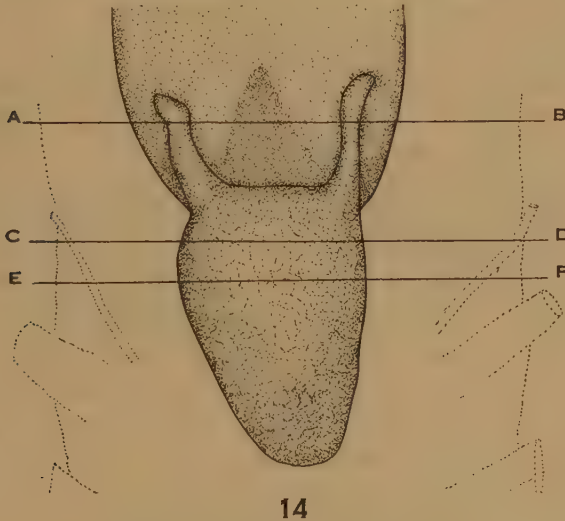


Fig. 14 Camera-lucida drawing showing a ventral view of the hypophysis of an adult of *Necturus maculosus*, caudal end below. A-B, C-D, and E-F, indicate planes of sections shown in figures 15, 16, and 17, respectively. $\times 15$.

the adult in figure 13. Figure 12 presents a sagittal section of the adult hypophysis. It may be seen that the pars intermedia is relatively large. The anterior lobe proper is small and is situated almost entirely caudal to the infundibulum. Corresponding to the large size of the pars intermedia the neural lobe is also relatively large. The two components of the pars tuberalis maintain their attachment to the anterior lobe proper, even in the adult stage (fig. 13).

c. The hypophysis of Necturus

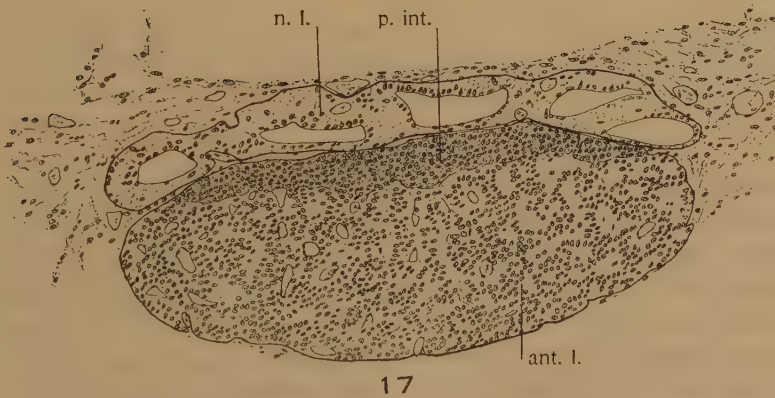
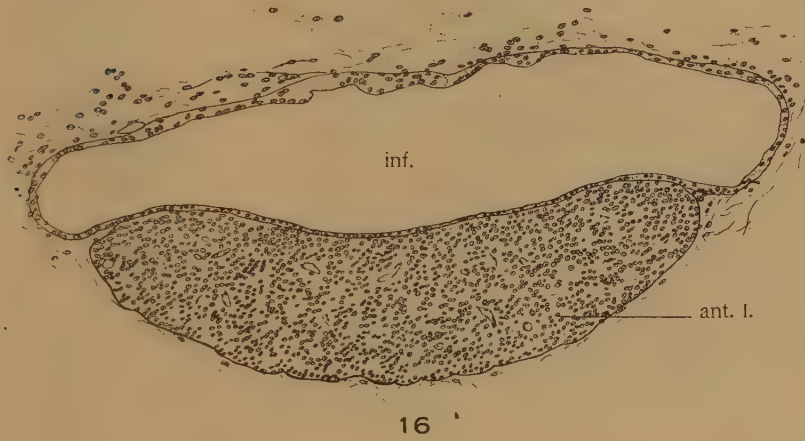
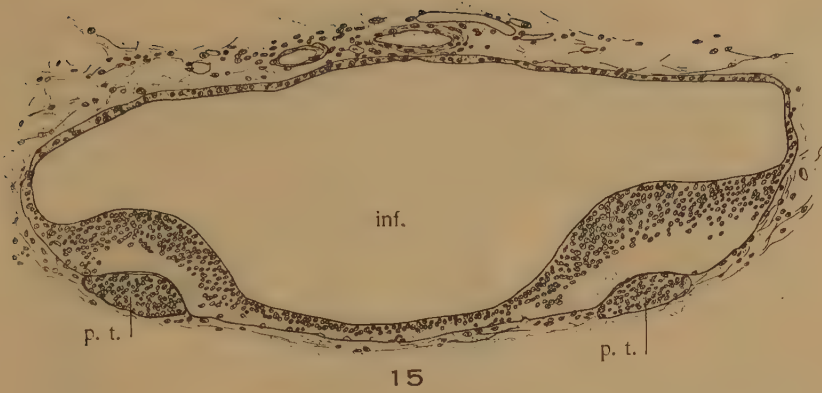
A camera-lucida sketch of the hypophysis and adjacent brain floor in an adult is given in figure 14. The lines *A-B*, *C-D*, and *E-F* indicate the positions of the transverse sections shown in figures 15, 16, and 17, respectively. As may be seen from figure 14, the two portions of the pars tuberalis remain united to the anterior lobe. The latter is elongated with its greatest dimension extending anteroposteriorly.

Figure 15 shows a transverse section through the pars tuberalis (fig. 14, *A-B*), which is seen as two epithelial strands lying under thickenings in the floor of the diencephalon. Between these two thickenings the infundibular floor is very thin. A more caudal section is shown in figure 16 (fig. 14, *C-D*). Here only the thin-walled infundibulum and the anterior lobe proper may be seen. A section somewhat farther caudalward is given in figure 17. The neural lobe, the pars intermedia, and the anterior lobe proper are seen. The neural lobe is very much sacculated, so that the infundibular cavity is cut five times in this one section. The pars intermedia is a thin strip situated between the other two parts. It is not very vascular in comparison with the anterior lobe; its nuclei are more closely crowded together, and with the commoner stains it is considerably darker in appearance. The anterior lobe contains large, thin-walled blood spaces and its cells have a cord-like arrangement.

d. The hypophysis of Amphiuma

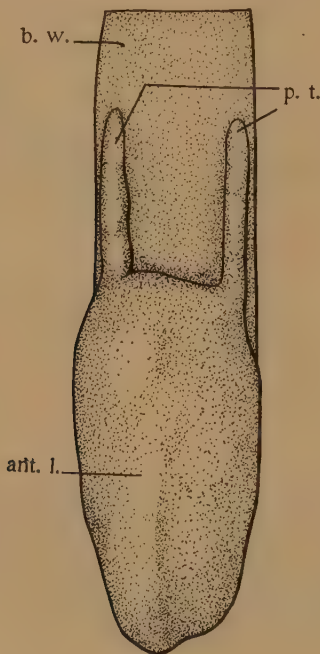
Sections and a wax-plate reconstruction from adults of *Amphiuma* means (figs. 18 and 19) show the same general features as seen in the other tailed amphibia studied. Certain distinguishing features may be noted, however. The gland appears to be flattened from side to side (fig. 18) and much thicker in a dorsoventral dimension (fig. 19). In the latter respect the difference is marked when compared with *Spelerpes*. Also in *Amphiuma* the anterior lobe is relatively greater in bulk.

The pars intermedia is compact in structure and dark staining (fig. 19). The neural lobe is well sacculated, as in *Necturus*.



Figs. 15, 16, and 17 Transverse sections of the hypophysis of an adult *Nec-turus*, shown in the gross in figure 14. The planes of the sections are shown by the lines *A-B*, *C-D*, *E-F*, figure 14, respectively. $\times 50$.

The pars tuberalis, as in the other forms studied, maintains its connection with the nasal end of the anterior lobe (fig. 18).



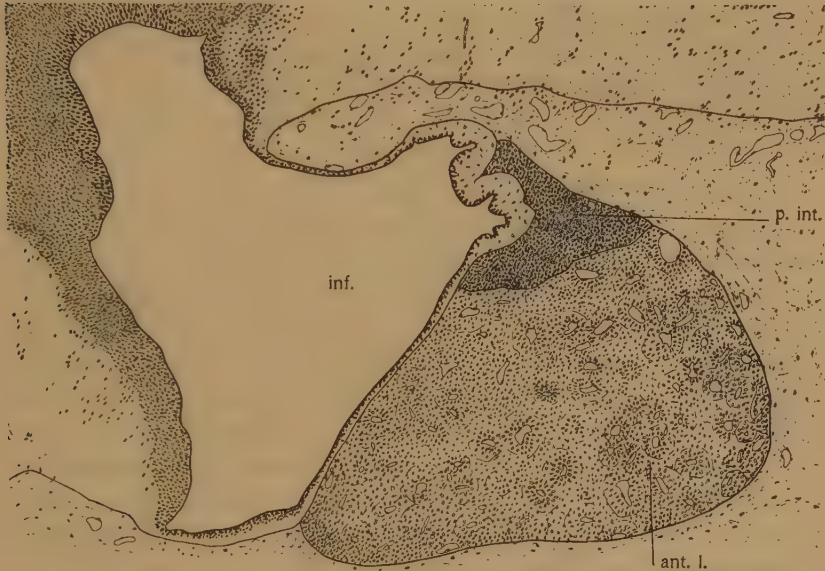
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Fig. 18 Ventral view of a wax-plate reconstruction of the hypophysis of an adult *Amphiuma*, caudal end below. *b.w.*, brain wall; *p.t.*, pars tuberalis; *ant.l.*, anterior lobe proper. $\times 25$.

DISCUSSION

This study has not been primarily concerned with the early stages in the development of the hypophysis. All the evidence, however, goes to confirm the opinion that the gland is entirely ectodermal in origin, and that no contribution is made by the entoderm or the notochord, as has sometimes been claimed for the amphibia (Kupffer, '94, and Valenti, '95). The material studied has not given satisfactory evidence to confirm the statement of Kingsley and Thyng ('04) that the hypophysis has a bilateral origin in *Ambystoma*.

The hypophysis breaks loose from the ectoderm in amphibia a considerable time before the rupture of the oral plate. This is the reverse order from that obtaining in birds and mammals where the oral plate ruptures early and the hypophysis maintains its connection with the ectoderm until much later.



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Fig. 19 Midsagittal section of the hypophysis region of an adult *Amphiuma*. Nasal end at left. *inf.*, infundibulum; *p.int.*, pars intermedia; *ant.l.*, anterior lobe proper. $\times 30$.

That the two tongue-like processes attached to the nasal end of the anterior lobe are to be considered homologous with the pars tuberalis in the higher vertebrates seems to me very certain. They are similar in all respects to the processes seen in the larval stages of the anura (cf. Atwell, '18 a).

These structures have been noted by Stendell ('14), who describes them in these words:

Bei den Urodelen und Anuren kann noch ein besonderer Hypophysenteil unterschieden werden, der hier Erwähnung finden möge.

Er liegt im allgemeinen von den übrigen Hypophysenabschnitten, dem Haupt- und Zwischenlappen, getrennt weiter vorn fast unter dem Chiasma opticum, dem Hirnboden dicht angepresst (Fig. 43, 44 und 26c). Er ist durchaus drüsiger Natur und zum Darmteil gehörig. Meistens ist er paarig entwickelt in Form zweier symmetrisch zu beiden Seiten der Medianen gelegener, flach-linsenförmiger Zellhäufchen. Dieser Hypophysenteil ist Pars anterior, auch Pars chiasmatic agenannt worden.

Stendell is uncertain whether this pair of processes should be classed with the anterior lobe proper or the pars intermedia. He has not attempted to homologize it with the pars tuberalis, since the individuality of this lobe was not recognized by him for any of the vertebrate classes.

The most striking feature to be observed in comparing the hypophysis of the urodeles and the anura is that in the former the two tongue-like processes of the pars tuberalis do not become detached from the anterior lobe proper, even in adult life. It will be recalled that in the anura the processes become detached at the time of metamorphosis and form two discrete epithelial plaques. This peculiarity was noted by Stendell for at least one form. He states: "Ferner scheint die Hypophyse von *Proteus anguineus* in dieser Beziehung primitive Verhältnisse zu zeigen. Bei ihr nämlich bleibt jener vordere Teil zeitlebens als zungenförmige Verlängerung an dem Hauptlappen des Darmteils hängen."

That this condition is a constant one for the tailed amphibia seems certain, since it is also found to obtain in *Ambystoma*, *Spelerpes*, *Necturus*, and *Amphiuma*. It is interesting to speculate upon a possible relationship between this apparently 'primitive' condition of the hypophysis and the retention of the tail throughout adult life. Is there anything more than coincidence to be assigned to the fact that in the higher Amphibia when the tail is lost at metamorphosis, the pars tuberalis likewise becomes detached?

It must be noted that the peculiar detached condition of the pars tuberalis in the adult anura is not general for the higher vertebrate classes, since in birds and mammals the lobe is usually found connected with the remainder of the gland. It is

often detached, however, and sometimes disappears entirely in certain reptiles (Baumgartner, '16).

The sacculations of the neural lobe, noted in two of the forms studied, were not observed for the frog or the toad (Atwell, '18 a). In this respect the hypophysis of the tailed amphibia is the more closely related to that of the fishes, where in certain forms, notably the elasmobranchs, the sacculation of the neural lobe and its interdigitation with the pars intermedia are very extensive.

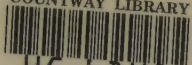
SUMMARY

In the tailed amphibia the epithelial hypophysis is developed from the ectoderm and differentiates into three lobes: the pars anterior proprior, the pars intermedia, and the pars tuberalis. The pars anterior proprior, or anterior lobe proper, forms the main bulk of the gland and comes to lie caudal and ventral to the infundibulum. The pars intermedia is developed from the dorsocaudal extremity of the early hypophysial fundament. In its adult position it lies caudal to the neural lobe and dorsal to the anterior lobe. The pars tuberalis develops from a pair of processes which grow forward from the remainder of the gland. These processes do not become detached to form separate epithelial plaques as in the anura, but maintain their connections with the anterior lobe throughout life. The neural lobe is considerably sacculated in *Necturus* and *Amphiuma*. Judging from this criterion alone, these two forms are primitive and are rather closely related to certain of the fishes.

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